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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Part V

DIVISION OF CANCER RESEARCH RESOURCES & CENTERS

United States.

NATIONAL CANCER INSTITUTE

ANNUAL REPORT of program activities

DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

July 1, 1975 through June 30, 1976

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OFFICE OF THE DIRECTOR
DIVISION OF CANCER RESEARCH RESOURCES AND CENTERS

Thomas J. King, Ph.D., Director

HISTORY AND CONTENT OF PROGRAM

The Division of Cancer Research Resources and Centers (DCRRC) administers research programs that encompass the full scope of laboratory and clinical cancer investigation, training, and education programs that develop the research and clinical personnel needed for the National Cancer Program, and a centers program that develops organizational resources and facilities to enhance the Nation's cancer research and control efforts. The Division emphasizes meritorious and significant cancer research, resource, and manpower initiatives conceived and promulgated by investigators outside the federal establishment. The research initiatives include cancer epidemiology, chemical and viral carcinogenesis, tumor biology, radiation biology, immunology, diagnosis, drug development, and cancer therapy.

The Division's National Organ Site Programs support targeted research on bladder, large bowel, prostatic, and pancreatic cancer. The Division fulfills its research and clinical personnel development responsibilities by making Research Career Development Awards, by awarding grants in the Clinical Cancer Education Program, and by providing National Research Service Awards for individual fellowships and for institutional programs. The Division addresses its responsibility in resource development by providing core support in Comprehensive Cancer Centers and in other centers of excellent cancer research by supporting exploratory and planning efforts and by providing grants for constructing laboratory and clinical cancer research facilities.

Research:
The allocation of resources to support regular research grants and program project grants attests to the National Cancer Institute's recognition of unfettered scientific thought, independently conceived initiatives, and individually planned and executed inquiry as most important elements of the National Cancer Program. Through the award of these grants, the Division of Cancer Research Resources and Centers emphasizes the value of intellectual contributions and unrestricted scientific endeavors of the Nation's scientists. Thus, within broad program guidelines, the Division draws upon the independent ideas embodied in unsolicited research proposals from investigators who represent the entire range from new scientists in the field to recognized authorities in all aspects of investigative oncology. Aided by peer evaluation of detailed proposals in every field of cancer inquiry, the Division supports research deemed most outstanding in regard to its significance for cancer, its conceptual base, its design, and its probable success.

This selection procedure, which is the mainstay of the Division's programs, constitutes the most sophisticated planning and implementing process available to the cancer effort. It begins with the broadest possible intellectual input and identifies and supports the ideas and approaches most likely to contribute to understanding or to managing cancer. This process is in

a constant state of reassessment in that it continually receives and evaluates new inputs which, in turn, are constantly influenced by the results of current research.

Organ Sites:
The Division's National Organ Site Program also leans heavily on the initiatives of the Nation's scientists. More than any other targeted effort of the Institute, the National Organ Site Projects look to non-Federal scientists for organized research endeavors. While administrative aspects are still evolving, the National Bladder, Large Bowel, and Prostatic Cancer Projects, are successfully underway. The start of the National Pancreatic Cancer Project was slowed by budgetary uncertainties that were resolved very late in the fiscal year.

Clinical Education:
The Clinical Cancer Education Program may be the most effective, economical, and efficient provider of people benefits of all the programs within NCI. By supporting improved cancer teaching in medical school curricula and in teaching hospitals, this program enhances the understanding of cancer for graduating M.D.'s. Dental students learn to detect cancer and to provide proper dental care to patients undergoing and recovering from cancer therapy. Even for students who are not influenced to choose careers in cancer research or cancer care, the program instills a better understanding of cancer than standard curricula provide. It will result in early cancer detection, improved diagnosis, timely referrals, and enhanced care during followup and rehabilitation. The program aims to provide a new generation of academicians and practitioners far better prepared than their predecessors to provide Americans with proper cancer care and treatment.

Research Manpower:
The Division develops cancer research talent through the support of institutional and individual fellowship and research career development awards. Through these awards the NCI provides research training in epidemiology, carcinogenesis, viral oncology, immunology, tumor biology, radiation, drug development, and chemotherapy. Once again these Research Manpower programs are subject to disruption and instability generated by delayed legislative authority, changing policies, and uncertain fiscal support. NCI, along with all of the NIH, entered the last quarter of FY 1976 without renewed legislative authority and without an appropriation for institutional and individual fellowship programs.

Centers:
The Centers Program devoted a great deal of effort during the year to developing methods for evaluating comprehensive cancer centers. The staff also reviewed and revised guidelines for applicants and for reviewers of applications for center support (Core) grants. In April 1976, the Ohio State University Cancer Research Center was recognized as a Comprehensive Cancer Center. Thus 15 Comprehensive Cancer Centers have been recognized since passage of the National Cancer Act of 1971. Three institutions were deemed Comprehensive before the Act. The 18 recognized Comprehensive Cancer Centers now include:

The University of Texas System Cancer Center	Houston, Texas
Roswell Park Memorial Institute	Buffalo, New York
Memorial Sloan-Kettering Cancer Center	New York, New York
Sidney Farber Comprehensive Cancer Center	Boston, Massachusetts
Johns Hopkins Oncology Center	Baltimore, Maryland
Duke University Comprehensive Cancer Center	Durham, North Carolina
University of Alabama Comprehensive Cancer Center	Birmingham, Alabama
University of Wisconsin Clinical Cancer Center	Madison, Wisconsin
Fred Hutchinson Cancer Research Center	Seattle, Washington
University of Southern California/LAC Cancer Center	Los Angeles, California
Comprehensive Cancer Center of the State of Florida	Miami, Florida
Mayo Comprehensive Cancer Center	Rochester, Minnesota
Yale University Comprehensive Cancer Center	New Haven, Connecticut
Georgetown University/Howard University Comprehensive Cancer Center	Washington, D.C.
Illinois Cancer Council	Chicago, Illinois
Colorado Regional Cancer Center, Inc.	Denver, Colorado
The Fox Chase Cancer Center (University of Pennsylvania)	Philadelphia, Pennsylvania
Ohio State University Cancer Research Center	Columbus, Ohio

CREG:
The Division coordinated the development of guidelines and continues to coordinate the implementation of Cancer Research Emphasis Grants (CREG). DCRRC supplies NCI's liaison with NIH and the Division of Research Grants and provides grants management for CREG. CREG is used to promote and support research identified and described in public announcements by the National Cancer Institute. Announcements designate the research desired, but approaches and methodology are left to the creativity of the extramural scientists who apply. In March 1976, the National Cancer Advisory Board considered the first 162 CREG applications, of which 43.8% were approved. The following table summarizes CREG awards in FY 1976.

CREG FY 1976

NCI Division	Number of Grants	Total Costs (Thousands of Dollars)
DCBD	6	469
DCCP	28	1,871
DCT	6	284
Total	40	2,624

Organization Changes:
In FY 1976, responsibility for administering and for review of grant-supported cooperative clinical groups was transferred from the Division of Cancer Research Resources and Centers to the Division of Cancer Treatment. Responsibility for administering the Cancer Control Grant Review Committee was transferred to the Division of Cancer Control and Rehabilitation.

Budget:
Once again budget allocations were delayed until mid-March. Thus, the availability of funds was not clarified until three quarters of the fiscal year were almost over. Furthermore, the budget process imposed fiscal uncertainties disproportionately upon the grants programs of NCI. The instability visited upon grantees and the resulting impairment of the Nation's most important cancer efforts cannot be measured.

When the allocations were finally made, the funds available to support the programs of DCRRC through June 30, 1976 amounted to $323,978,000. The distribution of those sums is as follows:

FY 1976 BUDGET DISTRIBUTION
(Thousands of Dollars)

Program		Total Costs
Traditional	$129,011	
Scientific Evaluation	860	
TOTAL REGULAR PROGRAM		$129,871
Core	36,893	
Exploratory	2,701	
TOTAL CENTERS SUPPORT		39,594*
Program Projects		87,518
Radiation Development		4,000
RCA	282	
RCDA	2,958	
TOTAL CAREER PROGRAMS		3,240
Bladder	4,220	
Large Bowel	5,765	
Prostate	3,052	
Pancreas	1,063	
TOTAL ORGAN SITE		14,100
Clinical Education		7,492
Fellowships		12,840
Graduate Training	3,600	
Clinical Training	1,723	
TOTAL TRAINING		5,323
Construction		20,000
TOTAL DCRRC		$323,978

* Includes $1,000 from Cancer Control.

With the funds available, the Division made 2,964 awards and 554 supplements in FY 1976. The following table shows how the awards were distributed among noncompeting projects, competing renewals, new projects,and supplements. The table also compares the amounts requested with the amounts approved and the amounts awarded.

FY 1976
(Thousands of Dollars)

	Requested		Approved		Awarded		Unfunded	
	#	$	#	$	#	$	#	$
Type 5 (Non-competing)	2161	$208,119	2161	$208,119	2161	$206,710	0	$ 1,409
Type 2 (Competing Renewals)	503	103,126	421	54,984	264	43,534	157	11,450
Type 1 (New)	2143	265,811	1450	118,970	539	50,768	911	68,202
Type 3 (Supplements)	(489)	20,922	(572)	24,321	(554)	22,966	(18)	1,355
Total	4807	$597,978	4032	$406,394	2964	$323,978	1068	$82,416

Staff:
During the year, 32 people left the staff of DCRRC. Of these, seven were transferred when responsibility for cancer control grants review was transferred to the Division of Cancer Control and Rehabilitation and when responsibility for cooperative clinical investigations was transferred to the Division of Cancer Treatment. At the close of the fiscal year, DCRRC's total staff numbered 166.

OFFICE OF PROGRAM PLANNING

John T. Kalberer, Jr., Ph.D., Program Planning Officer

The Office of Program Planning is located in the Office of the Director, DCRRC, and is responsible for the planning and evaluation of grant-supported programs administered by the Division.

The Office of Program Planning (OPP) integrates data from various sources within the Division in order to establish a broad overview of past trends and present and future needs. It also serves the National Cancer Institute (NCI) as a whole by maintaining a close working relationship with the Office of Program Planning and Analysis (OPPA) in the Office of the Director, NCI.

To help fulfill many needs of the Division, the OPP administers and manages contracts with outside firms and cooperates with the OPPA in several other contract-supported studies. Some tasks accomplished with the aid of these contracts follow.

The OPP, in conjunction with the Radiation Program, administered a contract with WSA Inc. to study all aspects of equipment development in the field of radiation therapy. Upon completion of this contract the following three excellent reports were submitted to DCRRC: (1) Summary of Physical and Operational Characteristics of Megavoltage Radiation Therapy Equipment, (2) Review of Projected Operational Characteristics of Equipment for High LET Radiation Therapy Clinical Service, (3) High Energy Neutron Radiation Therapy Equipment for Routine Clinical Use.

The OPP, in conjunction with the Cancer Centers Branch, continues to study some of the factors necessary for an effective and efficient Cancer Centers Program. With the help of a contract with JRB Associates, an extension of a previous study on possible Cancer Center locations was completed. The new study analyzed the effects of the following three factors: accessibility of community physicians to cancer centers; physicians' access to medical schools which are part of existing centers or may be a part of a center in the future; and population access to hospital beds.

A continuing contract with CDP Associates for program planning in the Cancer Centers Program called for:

- identification and delineation of the individual steps composing an evaluation planning process
- description of present and past evaluation and review processes and a recommendation of how these relationships can be upgraded
- identification of "urgent issues" that require immediate analysis and description of the potential approach to resolve each issue
- a summary of the experiences associated with the coordination of this planning effort and a recommendation of how this coordination can be improved.

RESEARCH ANALYSIS AND REPORTING BRANCH

Harry Y. Canter, Chief

HISTORY AND CONTENT OF PROGRAM

Under the recent reorganization of the Division of Cancer Research Resources and Centers (DCRRC), the Research Analysis and Reporting Branch (RARB), formerly the Program Analysis and Evaluation Branch, has been assigned to the Office of the Director of DCRRC. The major emphasis of the Branch remains the scientific analysis of all programs supported by DCRRC, all NCI contracts, and, to a limited extent, NCI intramural projects. The Branch serves as the major source of information concerning the scientific aspects of NCI-supported research projects, and as a point for dissemination of that information to other Divisions of NCI, the Office of Cancer Communications, the NCI Financial Management Branch, the NCI Program Analysis and Formulation Branch, the International Cancer Research Data Bank, and other NIH organizations such as the Research Documentation Section and the Research Analysis and Evaluation Branch of the Division of Research Grants (DRG), and other NIH Institutes.

The Branch maintains copies of grant applications and contract proposals for active projects, progress reports, Study Section summary statements, reprints of published grant-supported papers, and documents concerning individual trainees, fellows, etc. Administrative data, e.g., awarded amounts, active dates, etc., are maintained in order to indicate program emphasis and direction. However, it is the scientific research, both proposed and actually accomplished, which remains the major concern of this Branch.

Pending grants and contracts are reviewed to assure that there is no duplication or overlap between these two forms of NCI support. The new Cancer Research Emphasis Grants (CREG) may present some problems because of possible overlap if an investigator has all three forms of support (grant, contract, and CREG). However, it appears that most of the CREG work was formerly supported by contracts and will not overlap or duplicate grant support.

Proposed research described in applications of awarded grants is analyzed by the Branch's scientific staff, indexed, and stored in computer-searchable form. The Smithsonian Science Information Exchange (SSIE) abstracts supplied by the applicants are also stored in this system but must be retrieved by, for example, grant number or principal investigator's name, since they are not directly searchable. The NCI grant administrative data from DRG's IMPAC system are also stored in the Branch's system and can thus be linked to the scientific data in various combinations to produce printouts of the desired information in any of several available formats.

Accomplished research conducted under the various grants programs of DCRRC is monitored by the Branch through its literature surveillance program. Each issue of approximately 300 major medical and scientific journals is scanned to produce a list of references to papers resulting from NCI grant-

supported research. These references, supplemented by those obtained from reprints submitted by grantees, are arranged numerically by grant number within each Program Area and issued monthly, together with a subject index, as the NCI Grant Supported Literature Index. The Index represents the most complete record of accomplishments of the total extramural research programs. In calendar year 1975 approximately 3,900 articles, representing the work of 8,603 individual authors and acknowledging NCI grant support a total of 5,832 times, appeared in the Index.

Narrative reports on selected areas of cancer research are prepared by the Branch either at the request of DCRRC staff or on the initiative of Branch scientific staff members. Two reports written in early 1975, "The Role of Diet in Cancer Therapy" and "Dietary Factors in the Etiology of Cancer," have now been reproduced over 500 times, primarily at the request of the new NCI Task Force on Diet and Nutrition.

PROGRAM ACCOMPLISHMENTS

In April 1975, the Branch, with the aid of the NIH Division of Computer Research and Technology (DCRT), began automation of its Scientific Indexing System for funded NCI grants; this system, which now contains approximately one-third of the active grants, is replacing the edge-punch card system which has been in use for almost 20 years. The system, which is used to respond to queries and to prepare reports, is comprised of three types of data: administrative (from DRG's IMPAC System), scientific indexing, and SSIE abstracts. The Branch directly supplies only the scientific indexing, which is obtained by analyzing the entire grant application. Each grant is indexed according to a list of fixed terms compiled by the Branch; in addition, as many as 60 other, non-fixed key words and/or phrases can be used to index each grant, thus providing a specific and detailed subject analysis of the project.

The system, now termed the NCI Grants Information Retrieval System, is composed of two separate files: the active grants file and the inactive grants or history file. All scientific and administrative data on both files, except for the SSIE abstracts, are searchable. The NCI Grants Information Retrieval System is maintained and operated solely by the Branch, which is greatly indebted to DCRT for their help in developing and implementing the system, and for their assistance in making the Branch self-sufficient in the total operation of the system.

DCRT has also formulated programs to permit preparation of annual cumulations by subject, grant number, principal investigator, grantee institution, and author for the references cited in the monthly NCI Grant Supported Literature Index. They have also written a retrieval program allowing access to the total Reference Section of the Index, which is stored on tape.

Several subject analyses of NCI-supported research were prepared by the Branch during the past year; these included such topics as nucleoside research, cell kinetics, genetic recombinants, and the etiology, detection, and treatment of brain cancer. One of the most extensive analyses involved

the area of environmental carcinogenesis. This was requested by the National Cancer Advisory Board and included all DCRRC-supported projects (research grants, career awards, fellowships, and training grants).

Two narrative reports were prepared during the past year:
The Development of Chemotherapeutic Drugs
Chronobiology: Its Implications for Cancer Research

The Branch assisted the Cancer Communications Office in preparing the NCI Director's Report to the President for 1976. Members of the staff reviewed, selected, and reported on research supported by NCI in all basic and clinical disciplines related to cancer.

This year, for the first time since 1960, this Branch did not prepare a scientific narrative Appendix to the DCRRC Annual Report. Instead each Program Director expanded her/his own report to include a review of research published during the past year. The entire report has been edited by the Branch staff. It is hoped that the new format will provide easier access to information on all aspects of each program of the Division.

PROJECTIONS

It is anticipated that the NCI Grants Information Retrieval System will be expanded before the start of FY 1977 to include a third file, consisting of disapproved and approved-but-not-funded grants. This file will contain only administrative data, with project title search as the only point of subject access. A similar interim file will be set up for active contracts until scientific indexing of active research grants has been completed. When the latter has been accomplished, comprehensive scientific indexing of the contracts will be undertaken according to the format now used for grants (fixed terms plus key words and/or phrases).

OTHER ACTIVITIES

The Branch maintains regular liaison with other Divisions of NCI. Extra copies of reprints of grantee papers are sent to each Division, as are copies of the SSIE abstracts for all grants. SSIE abstracts of grants concerned with specific research areas are sent on a regular basis to NCI staff who have requested this service.

Staff members also serve on Divisional and Institute Committees (e.g., Steering Committee of the Breast Cancer Task Force) which attempt to foster better communication concerning NCI-sponsored research and to promote research in areas judged to be of special significance to the National Cancer Program.

REVIEW AND REFERRAL BRANCH

Thomas J. King, Ph.D., Acting Chief

During the FY 76, the Review and Referral Branch, DCRRC, was responsible for providing the merit review of grant applications seeking support for cancer research program projects, comprehensive and specialized cancer centers, and construction projects. The Review and Referral Branch also provided the Division's liaison with the Referral Branch of the Division of Research Grants, NIH, as well as the referral function within the Division.

The Review and Referral Branch administers and staffs two multidisciplinary review committees: the Cancer Research Centers Review Committee (CRCRC) and the Cancer Special Program Advisory Committee (CSPAC). A small number of applications are reviewed by ad hoc Special Review Committees (SRC). This is usually necessary because of the NIH policy prohibiting a Committee from reviewing a proposal from one of its members. CRCRC and CSPAC review grant applications for program projects, centers, and construction. The core section of CRCRC reviews core grant applications for comprehensive cancer centers and for predominantly clinical specialized centers that are developing into comprehensive cancer centers. The clinical section of CRCRC reviews applications for research program projects with predominantly clinical emphasis. CSPAC reviews core grant applications and research program project applications that empasize laboratory and basic research. During FY 76, these committees evaluated 141 of these complex applications. The number of applications, the dollars requested, and dollars recommended by each committee are summarized in the table below.

Initial Review Group	Requested		Recommended		Approval Rate	
	#	$	#	$	#	$
CRCRC-Core	29	85,818,701	22	34,407,691	76%	40%
CRCRC-Clinical	44	198,907,701	31	56,454,442	70	28
CSPAC	60	127,081,046	37	37,307,193	62	29
SRC	8	23,008,719	8	9,481,241	100	41
Total	141	434,816,167	98	137,650,567	70%	32%

The referral responsibility of the Review and Referral Branch is illustrated by its activities related to applications received for review by the National Cancer Advisory Board in March 1976. Some of the applications assigned to the National Cancer Institute were "automatically" assigned to particular programs such as the National Organ Site Program, the Clinical Cancer Education Program, and the Cooperative Clinical Investigations Program. However, a total of 777 applications required individual assignment to cancer programs. Of this total, 672 were traditional research projects, 34 were research program projects, seven were center cores, 13 were conferences,

and 51 were research career development awards. In addition, for the first time, 173 applications were received for Cancer Research Emphasis Grants (CREG). These applications were coordinated with the Division of Research Grants and the various Cancer Institute Divisions through the Review and Referral Branch based on responsiveness to individual CREG announcements.

BIOLOGICAL RESEARCH PROGRAM

Mary A. Fink, Ph.D., Associate Director

The Biological Research Program of the Division of Cancer Research Resources and Centers of NCI is comprised of the programs of three branches: the Cause and Prevention Branch, made up of programs in Chemical Carcinogenesis, Viral Oncology, and Epidemiology; the Cancer Biology Branch, comprised of programs in Tumor Biology and Immunology; and the Organ Site Branch, including programs in cancer of the Large Bowel, Bladder, Prostate, and Pancreas. Program directors are encouraged to initiate special interest groups where many program directors participate, even though the Division has no formal program director in that area, e.g., genetics as it applies to cancer is a field which interests the program directors for Tumor Biology, Immunology, and Epidemiology.

Program directors are encouraged to maintain program balance but at the same time to develop areas that have been identified as being of special importance. One area currently receiving Institute emphasis is environmental carcinogenesis, and the program director of the Carcinogenesis Program is vigorously addressing this need.

In addition to relating to other programs within the Division, many of the programs participate in counterpart contract programs in other divisions of the Institute. For example, the program directors for Viral Oncology, Carcinogenesis, Immunology, and Epidemiology regularly participate in contract meetings. The Immunology Program also cosponsors an annual meeting in which individuals holding either a grant or contract in a particular area of immunology meet together to discuss the current research.

The Biological Research Programs funded 13 conference grant applications in FY 76. In addition, two productive workshops-- one on the Macrophage in Tumor Immunity and the other, Immune RNA in Tumor Immunity-- were held, and the proceedings of each will be published in 1976 under the editorship of the Associate Director for Research Programs. Also, as mentioned in the Immunology report, two regional conferences were held for promising young investigators in an attempt to attract them into the area of tumor immunology. Program directors are encouraged to identify areas where increased communication would be beneficial and, in consultation with investigators in the field, develop workshops and conferences addressing the most pressing needs.

TUMOR BIOLOGY PROGRAM

Brian W. Kimes, Ph.D., Program Director

HISTORY AND CONTENT OF PROGRAM

The Tumor Biology Program was created in March of 1974 as a separate program activity within the Division of Cancer Research Resources and Centers. The original goal was to establish a fundamental understanding of living systems which potentially could be used to enhance the overall cancer research effort as it relates to etiology, prevention, diagnosis, and treatment of human tumor growth and development. Although this program continues to support a comprehensive spectrum of research topics, including both fundamental and comparative studies in histology, pathology, cell biology, embryology, molecular biology, biochemistry, genetics, and developmental biology, a greater focus in its activities is emerging. The goals of this program are to promote an understanding of the basic processes of cancer and to define the causative events in tumorigenesis which lead to and/or are responsible for disarrangement of cell topology (loss of position control), persistent growth (loss of growth control) and invasiveness (a prerequisite for metastasis). Major emphasis areas which are currently in progress and require a multidisciplinary scientific effort include the following:

(1) the mechanism(s) and regulation of cell division;
(2) the mechanism(s) of malignancy, invasiveness, and metastasis;
(3) factors (whether genetic, hormonal, etc.) which contribute to the resistance and susceptibility to cancer;
(4) the mechanism(s), regulation, and control of macromolecular biosynthesis and degradation (RNA, DNA, protein);
(5) the mechanism(s), regulation, and control of synthesis of small biomolecules (metabolism, metabolic pathways etc.);
(6) the correlation of normal and abnormal differences in cell structure and and composition to function;
(7) the nature of cell-cell communication and interactions.

Fundamental studies supported by the Tumor Biology Program are intended to elucidate the nature of basic processes important to the structure and function of normal and/or eukaryotic cells. These studies need not specifically involve tumor tissues or tumor systems since they are intended to develop areas of investigation with an identifiable contribution to the goals of cancer research in terms of concepts, methodology, technology, and/or experimental design. Comparative studies are intended to define cellular processes, structures, and components that differ between normal and abnormal states of growth, between normal tissues and their neoplastic counterparts, between nontransformed and virally transformed cultured cell lines, between progressive states of differentiation and development, and between different phases of the cell growth cycle. It is anticipated that these latter kinds of studies will provide substantive information defining specific cellular sites of action and molecular mechanisms that are causal to tumor growth and development and thus lead to practical therapeutic and preventive measures. Furthermore, differences detected between normal tissues and their neoplastic counterparts can offer potentially useful and economical diagnostic tools to the medical practitioner.

In FY 1976, the Tumor Biology Program consisted of 365 traditional research grants, five conference grants and seven program project grants at an estimated expenditure of $26.023 million distributed as follows:

TABLE A

TOTAL GRANTS FUNDED IN FY 76

TYPE OF GRANT	NUMBER	TOTAL COSTS
Traditional Research Grants		
New (Type 1)	63	$ 3,490,000
Competitive Renewals (Type 2)	48	3,840,000
Supplements (Type 3)	(11)	306,000
Continuing Renewals (Types 5,7)	254	15,410,000
TOTAL	365	$23,046,000
Conference Grants	5	$ 140,000
Program Projects		
New (Type 1)	1	$ 143,000
Continuing Renewals (Type 5)	6	2,694,000
TOTAL	7	$ 2,837,000
GRAND TOTAL	377	$26,023,000

RESEARCH ACCOMPLISHMENTS

During the past year, significant contributions were made toward elucidating the molecular basis of cellular processes and control mechanisms which influence the growth and differentiation of malignant and nonmalignant cells.

Membrane Structure and Function:
The most important mammalian cell structure involved in the complex interactions with the environment and with other cells is the plasma membrane (i.e. cell surface membrane). Phenotypically, changes in the structure and composition of the cell membrane may account for all three basic processes characteristic of malignant tumor cells: loss of position control, loss of growth control, and invasiveness. A revised Fluid Mosaic Model of membrane structure (Nicolson, 1975a,b) not only emphasizes the dynamic fluid nature of the plasma membrane but includes new facts which demonstrate that the inner surface peripheral membrane components (Furcht and Scott, 1975) and membrane-associated

contractile components (Wickus et al., 1975; Gruenstein et al., 1975) provide two additional levels of membrane organization on the cytoplasmic side of the plasma membrane. These latter classes of membrane components appear to exert control over topographical distribution and mobility of cell-surface components. For example, actin associated with the inner surface of the fibroblast cell membrane is considerably reduced (30-50%) after transformation by Rous sarcoma virus (Wickus et al., 1975). Because this transformation is also accompanied by changes in cell surface glycolipids and glycoproteins as indicated by lectin-mediated agglutinability of cells (Nicolson et al., 1975), turnover of phospholipids, membrane transport, and contact inhibition, it may be that all these phenomena are related to membrane-associated actin which serves as a major factor in regulating membrane structural rigidity. This new fact may be of vital importance in understanding malignancy, as changes in membrane rigidity (i.e. cell deformability) are likely to be a major factor contributing to invasiveness and metastasis. Before a cell can be metastatic by entering the blood stream, it first must become invasive and migrate through narrow intercellular spaces.

In order to understand the structure, organization and biochemistry of the surfaces of normal and tumor cells and their relationship to important physiological processes, a number of different experimental approaches are required. Recently, it has been shown that increased negativity of neuroblastoma cell surfaces, as determined by electrophoretic measurements, is clearly associated with cell proliferation rather than differentiation (Elul et al., 1975). Since glycoproteins appear to contribute significantly to this negative charge, it has been of interest to examine the biosynthesis of glycoproteins by using radioactive fucose as a precursor. GDP-fucose appears to be the only soluble fucose intermediate in the cytoplasm (Yurchenco and Atkinson, 1975) and newly synthesized glycoproteins containing fucose move to the plasma membrane with a transit time of only 12 minutes (Atkinson, 1975). The distribution and mobility of these anionic sites on the fluid surfaces of BHK cells appear to be more localized on cell surface microextensions when measured by polycationic ferritin binding (Grunnel et al., 1975). Yet, the mobility of these sites, especially between normal and transformed cells, does not appear to be due to significant changes in fatty acid chain flexibility in the membranes (Gaffney, 1975). A significant advance has occurred in the isolation of Chinese hamster ovary cells which are deficient in certain plant lectin-binding sites and have decreased amounts of sialic acid, galactose, and N-acetyl glucosamine in their membranes (Gottlieb et al., 1975). It is now possible with this cell line to examine what effects the absence of certain glycoproteins have on such processes as virus infection, cellular aggregation, tumorigenicity, and drug sensitivity. While the interrelationships of these observations remain unclear, it is essential to assess the role of surface proteins in membrane function as well as in the regulation of DNA synthesis and cell proliferation (Teng and Chen, 1975).

Cell-cell interactions and cell-cell communication both appear to be important factors that change when a cell becomes oncogenic. It has been pointed out by Steinberg (1975) that many of our current concepts of cell adhesion are based solely on common chemical mechanisms and do not take into account physical and thermodynamic considerations, which can be equally important in predicting cell-sorting behavior. Recently, it has been demonstrated, using gyratory

shaker cultures which can be controlled and standardized, that embryonic cell aggregation can be significantly reduced (greater than 22%) in the presence of a proportionally smaller number of virally transformed 3T3 cells (Maslow and Mayhew, 1975). From all indications these effects were mediated by the cell surface; thus, this could serve as a sensitive assay for investigating the interaction of tumor and normal cells. A protein-containing molecule, teratoma cell adhesion factor (TAF), has been isolated; it mediates cell-cell adhesion in mouse ascites teratoma cells (Oppenheimer, 1975). Whether this factor has a more universal effect is unknown, but it has been determined that a galactose-containing carbohydrate in TAF has aggregation promoting activity.

More information on how direct cytoplasmic communication between adjacent cell interiors occurs through gap junctions continues to accumulate (Johnson et al., 1975; Ito et al., 1974a,b). Some low resistance coupling (presumably due to gap junction formation) is observed as early as eight to 14 minutes after cell contact, and by 60 minutes numerous fully-developed structural gap junctions appear. Unfortunately, very little is being done to isolate these structures and directly determine their effects on homeostasis and the regulation of growth and differentiation.

Control of Gene Expression:

While many of the phenotypic properties of malignant and benign tumors are indirectly associated with membrane properties, ultimately oncogenicity must arise because of a change in the expression of the native genetic material (e.g. by mutagenesis, by "turning on" of a masked gene) or because of the presence of foreign genetic-material (e.g. viral transformation). These changes in expression could occur at the level of replication, transcription, translation and/or translocation. In the mouse myeloma cell lines, giesma-banding techniques have revealed the possibility that loss of oncogenicity could be due to chromosome rearrangements or the presence of additional heterochromatin (Shepard et al., 1974). While these studies do not eliminate a more complex balance of chromosome functions as responsible for loss of oncogenicity, it remains essential to identify those chromosomes and eventually those specific genes which are responsible for tumorigenesis and which distinguish benign and malignant cells.

There appear to be many different mechanisms by which the transcription of DNA sequences in chromatin into complementary RNA sequences can be controlled. The highly basic histone proteins which are bound to DNA in eukaryotic cells are not present in sufficient variability to have specific effects on all genes. However, they clearly serve a more general role in regulating gene expression, possibly by altering chromosome structure for entry of RNA polymerase. Careful techniques have been developed which confirm that the distribution of histones on replicating chromosomes is entirely random (Jackson et al., 1975) and, furthermore, through fixation of the chromatin complex with formaldehyde and glutaraldehyde, that these histones are organized as fairly extensive linear overlapping arrays (Chalkley and Hunter, 1975). But what role do chemical modifications (i.e. acetylation, phosphorylation, methylation) of nucleoproteins serve in gene expression? Studies continually suggest that these modifications are closely correlated with the replicating state of tumor cells (Lee et al., 1975; Smoron et al., 1975; Balhorn et al., 1975). Yet the true biological functions of histone modifications can be explained in at least three different

ways: (a) gene activation, (b) histone deposition, and (c) chromosome condensation. Recent evidence suggests that (b) is important (Jackson et al., 1975).

The non-histone proteins associated with the DNA in chromatin appear to have the variability required for specific gene control. Recently, a special class of nonhistone protein has been identified which is very tightly complexed to the DNA in regions that are inactive in transcription (Pederson and Bhorjee, 1975). It will be interesting to determine the specific physiological role that these proteins serve in gene expression, if any, and determine the DNA sequences with which they are associated. In addition to non-histone proteins, attention has been redirected toward low molecular weight RNA species in chromatin as having the necessary diversity to function as specific gene regulators (Marzluff et al., 1975).

A novel modification of both histone and non-histone proteins, which could easily have an influence on both replication and transcription, is poly ADP-ribosylation. Furthermore, this may be a major route by which phosphate is incorporated into nuclear proteins. This process requires both DNA and histones (and possibly non-histones), which appear to act in a synergistic manner, and parallels the rate of DNA synthesis in the HeLa cell cycle (Robert et al., 1975). It is clear that the degree of complexing between DNA and chromosomal proteins and the ability of poly ADP-ribosylation of nuclear proteins to release this template restriction and expose DNA primer sites change considerably during the growth cycle (Smulson et al., 1975).

A number of studies have pointed to an important role for cAMP and cGMP in malignancy. Recently, it has been demonstrated that cAMP levels change significantly in preneoplastic states of the thymus (Kemp et al., 1975) and that cAMP has a marked effect on stimulating DNA synthesis in liver (Short et al., 1975). It has been suggested that malignancy per se is associated with a low effective level of cAMP, which is a function not only of cAMP concentration but also of the level of the various proteins which bind cAMP and of the activity of its antagonists such as cGMP (Goldberg et al., 1975). There is increasing evidence that all cAMP-mediated changes of metabolic activities involve the phosphorylation of enzymes and other key proteins. Results disclosed by Farron-Furstenthal (1975) provide evidence that a) actively growing tissues contain a higher percentage of their total protein kinase activity in the nucleus than the parent nongrowing organ, and that b) the cytoplasmic protein kinase in hepatoma and fetal liver is less sensitive to regulation by cAMP than it is in adult liver. In any case, it remains important to determine how and where these cyclic nucleotides act in regulating gene expression, especially in proliferating tumor cells.

Other new information emphasizes the extreme complexity of eukaryotic cells and the many different sites in the cell where gene expression can be altered or disrupted. The nuclear membrane, for example, contains a nuclear pore complex which could serve many different functions in nucleocytoplasmic communication and/or organization of the interphase chromatin (Aaronson and Blobel, 1974). Clearly, abnormal translocation of mRNA from the nucleus to the cytoplasm could result in aberrant cell behavior. Whatever the role of nuclear pore complexes, it appears that they do not require membrane for either attachment to the nucleus or for maintenance of their structural integrity, that they are attached

to the lamina, and that they are composed of a few polypeptides and very little DNA, RNA and phospholipid (Aaronson and Blobel, 1975). It is envisioned that mRNAs are translocated as complexes with special proteins through these pores. Phosphorylation of these proteins could play a regulatory role in protein synthesis, mRNA storage, or mRNA translocation through the nuclear envelope to the cytoplasm (Auerback and Pederson, 1975). An interesting hypothesis has been offered concerning the synthesis of membrane proteins and transfer of the newly synthesized nascent polypeptide chain across the membrane. Recent observations have supported the "signal hypothesis" which states that a signal sequence of amino acids on the end of the nascent chain promotes transfer into and/or through the membrane (Blobel and Dobberstein, 1975a,b). If this system were altered or obstructed, the surface properties of cells could change significantly.

Growth Control by External Factors:
Growth of normal cells can be influenced by interactions of these cells with various polypeptide growth factors, including hormones, present in the surrounding fluids. One hypothesis is that as normal cells become more and more crowded they require higher and higher concentrations of growth factors in order to respond. Transformed cells, whether malignant or benign, appear to escape from normal growth controls by requiring less of these hormones and growth factors (Holley, 1975). Factors in serum may be vital components in releasing tumor dormancy, since vascular penetration (i.e. angiogenesis) often removes physiological limitations on size, permitting renewed logarithmic growth (Auerbach et al., 1975). Previous work indicates that cell survival, cell migration, and cell multiplication are under the control of different serum factors. Recently, Wolf et al. (1975) have shown that a factor in rat plasma which promotes the initiation of DNA synthesis in confluent mouse 3T3 fibroblast cells is distinct from the factor that promotes cellular multiplication. While serum factors and components are important, available growth surface area also appears to limit cell growth (Thrash and Cunningham, 1975). Mitogenic activity has been observed for thrombin (Chen and Buchanan, 1975), for a cationic polypeptide from human serum (Antoniades et al., 1975) and mouse epidermal growth factor (Carpenter et al., 1975). Inhibition of plasma cell tumor growth by cAMP can be prevented by the addition of insulin to the culture medium (Nasum and Hollander, 1975) and it has been suggested that insulin resembles peptides in plasma which control or moderate cAMP transport effects. Liver and hepatoma extracts show growth inhibitory activity toward tumor cells which is proportional to their arginase activity (Nelson et al., 1975).

Information concerning the biological effects of these various stimulatory and inhibitory factors remains fragmentary and unclear; however, since most of these factors are present in blood serum, it would appear that once their mechanisms of action are elucidated, a unified theory can be constructed with potential application to clinical control of tumorigenesis and invasiveness.

Model Systems:
Fundamental biological research on tumorigenesis requires the use of model systems that can be clearly translated into an understanding of cancerous processes in humans. A number of potential model systems have emerged in the last year:

(1) a sensitive system for examining the interaction of tumor and normal cells (Maslow and Mayhew, 1975);
(2) a quantitative measure of the survival of invasive and/or circulating malignant tumor cells in vitro (Fidler, 1975);
(3) a quantitative way of evaluating changes in the growth rates of solid tumor models (hepatomas) after a series of radiation doses (Looney et al., 1975).

Diagnosis and Therapy:
A number of fundamental comparative studies conducted with normal and tumor cells or tumor-bearing animals and patients reveal differences that may have diagnostic and/or therapeutic clinical potential. For example, it has been shown that the sera from normal subjects and cancer patients show different galactosyl transferase activities. The majority of this enzyme activity migrated in an electrophoretic field as a broad peak just behind an albumin standard, but in cancer patients there was a new, slower moving isoenzyme activity (Podolsky and Weiser, 1975).

In rats bearing Morris hepatoma 3924A, the urinary excretion of cGMP, but not cAMP, increased significantly. The increased excretion of cGMP was diminished with local irradiation of the tumor, 5-fluorouracil administration, or tumor excision. Therefore, it appears that cGMP excretion can be used as an index of tumor growth and regression, although the universality of this observation will not be known until animals and patients with other kinds of tumors are examined (Mural et al., 1975). The Morris hepatoma 3924 in rats has also been used to note differences between the trace element levels in sera from 5-fluorouracil-treated and non-treated animals. Fe, Co, Rb, Cs, Se, and Ag in sera and in tumors change in parallel and these changes can be correlated to the number of 5-FU-blocked cells. These changes (Brookhart et al., 1975), like those noted above, may also prove to be valuable as a means of monitoring the effect of therapy.

REFERENCES

Aaronson, R.P. and Blobel, G. "On the Attachment of the Nuclear Pore Complex," J. Cell Biol. 62:746-754, 1974. (CA-12413)

Aaronson, R.P. and Blobel, G. "Isolation of Nuclear Pore Complexes in Association with a Lamina," Proc. Nat. Acad. Sci. 72:1007-1011, 1975. (CA-12413)

Antoniades, H.N., Stathakos, D. and Scher, C.D. "Isolation of a Cationic Polypeptide from Human Serum that Stimulates Proliferation of 3T3 Cells," Proc. Nat. Acad. Sci. 72:2635-2639, 1975. (CA-15388, CA-13472, CA-06516)

Atkinson, P.H. "Synthesis and Assembly of HeLa Cell Plasma Membrane Glycoproteins and Proteins," J. Biol. Chem. 250:2123-2134, 1975. (CA-13402, CA-06576)

Auerbach, R., Arensman, R., Kubai, L. and Folkman, J. "Tumor-induced Angiogenesis: Lack of Inhibition by Irradiation," Int. J. Cancer 15:241-245, 1975. (CA-14019, CA-13548)

Auerbach, S. and Pederson, T. "Phosphorylation of m-RNA-bound Proteins in HeLa Cells," Biochem. Biophys. Res. Comm. 63:149-156, 1975. (CA-13400, CA-12708)

Balhorn, R., Jackson, V., Granner, D. and Chalkley, R. "Phosphorylation of the Lysine-Rich Histones throughout the Cell Cycle," Biochemistry 14:2504-2511, 1975. (CA-10871, CA-12191)

Blobel, G. and Dobberstein, B. "Transfer of Proteins Across Membranes I," J. Cell Biol. 67:835-851, 1975a. (CA-12413)

Blobel, G. and Dobberstein, B. "Transfer of Proteins Across Membranes II," J. Cell Biol. 67:852-862, 1975b. (CA-12413)

Brookhart, W., Allen, R., Looney, W., Hopkins, H., Kovacs, C. and Morris, H. "A Study of Trace Elements in the Solid Tumor Model 3924A Hepatoma Before and After Treatment with 5-Fluorouracil," Life Science 17:233-240, 1975.

Carpenter, G., Lembach, K.J., Morrison, M.M. and Cohen, S. "Characterization of the Binding of 125 I-labeled Epidermal Growth Factor to Human Fibroblasts," J. Biol. Chem. 250:4297-4304, 1975. (CA-12810, CA-13534)

Chalkley, R. and Hunter, C. "Histone-Histone Propinquity by Aldehyde Fixation of Chromatin," Proc. Nat. Acad. Sci. 72:1304-1308, 1975. (CA-10871)

Chen, L.B. and Buchanan, J.M. "Mitogenic Activity of Blood Components. I. Thrombin and Prothrombin," Proc. Nat. Acad. Sci. 72:131-135, 1975. (CA-02015)

Elul, R., Brons, J. and Kravitz, K. "Surface Charge Modifications Associated with Proliferation and Differentiation in Neuroblastoma Cultures," Nature 258:616-617, 1975. (CA-13538)

Farron-Furstenthal, F. "Protein Kinases in Hepatoma, and Adult Liver of Rat I. Subcellular Distribution," Biochem. Biophys. Res. Comm. 67:307-314, 1975. (CA-16033)

Fidler, I.J. "Biological Behavior of Malignant Melanoma Cells Correlated to Their Survival in vitro," Cancer Res. 35:218-224, 1975.

Furcht, L.T. and Scott, R.E. "Effect of Vinblastine Sulfate, Colchicine and Lumicolchicine on Membrane Organization of Normal and Transformed Cells," Exp. Cell Res. 96:271-282, 1975. (CA-13458)

Gaffney, B.J. "Fatty Acid Chain Flexibility in Membranes of Normal and Transformed Fibroblasts," Proc. Nat. Acad. Sci. 72:664-668, 1975. (CA-14142, CA-15997)

Goldberg, M.L., Burke, G.C. and Morris, H.P. "Cyclic AMP and Cyclic GMP Content and Binding in Malignancy," Biochem. Biophys. Res. Comm. 62:320-327, 1975. (CA-10729)

Gottlieb, C., Baenziger, J. and Kornfeld, S. "Deficient Uridine Diphosphate-N-Acetylglucosamine: Glycoprotein N-Acetylglucosaminyltransferase Activity in a Clone of Chinese Hamster Ovary Cells with Altered Surface Glycoproteins," J. Biol. Chem. 250:3303-3309, 1975.

Grinnel, F., Tobleman, M.Q. and Hackenbrock, C.R. "The Distribution and Mobility of Anionic Sites on the Surfaces of Baby Hamster Kidney Cells," J. Cell Biol. 66:470-479, 1975. (CA-14609)

Gruenstein, E., Rich, A., Weihung, R.W. "Actin Associated with Membranes from 3T3 Mouse Fibroblast and HeLa Cells," J. Cell Biol. 64:223-234, 1975. (CA-12708, CA-04186)

Holley, R.W. "Control of Growth of Mammalian Cells in Cell Culture," Nature 258:487-490, 1975. (CA-11176)

Ito, S., Sato, E. and Loewenstein, W.R. "Studies on the Formation of a Permeable Cell Membrane Junction I," J. Membrane Biol. 19:305-337, 1974a. (CA-14464)

Ito, S., Sato, E. and Loewenstein, W.R. "Studies on the Formation of a Permeable Cell Membrane Junction II," J. Membrane Biol. 19:339-355, 1974b. (CA-14464)

Jackson, V., Shires, A., Chalkley, R. and Granner, D.K. "Studies on Highly Metabolically Active Acetylation and Phosphorylation," J. Biol. Chem. 250:4856-4863, 1975. (CA-10871, CA-12191)

Jackson, V., Granner, D.K. and Chalkley, R. "Deposition of Histones on Replicating Chromosomes," Proc. Nat. Acad. Sci. 72:4440-4444, 1975. (CA-10871)

Johnson, R., Hammer, M., Sheridan, J. and Revel, J.P. "Gap Junction Formation Between Reaggregated Novikoff Hepatoma Cells," Proc. Nat. Acad. Sci. 71: 4536-4540, 1974. (CA-11114)

Kemp, R.G., Hsu, P.Y. and Duequesnoy, R.J. "Changes in Lymphoid Cyclic Adenosine 3':5' - Monophosphate Metabolism During Murine Leukemogenesis," Cancer Res. 35:2440-2445, 1975. (CA-16539)

Lee, M.A., Koch, M.R. and Morris, H.P. "Nuclear Protein Changes in Rat Hepatomas Correlating with Growth Rate," Cancer Res. 35:1693-1697, 1975. (CA-10729, CA-12933, CA-16274)

Looney, W.B., Tregil, J.S. Schaffner, J.C., Kovacs, C.J. and Hopkins, H.A. "Solid Tumor Models for the Assessment of Different Treatment Modalities: I: Radiation-Induced Changes in Growth Rate Characteristics of a Solid Tumor Model," Proc. Nat. Acad. Sci. 72:2662-2666, 1975. (CA-12758, CA-13102, CA-10729)

Marzluff, W.F., White, E.L., Benjamin, R. and Huang, R.C.C. "Low Molecular Weight RNA Species from Chromatin," Biochemistry 14:3715-3724, 1975. (CA-13953)

Maslow, D.E. and Mayhew, E. "Inhibition of Embryonic Cell Aggregation by Neoplastic Cells," J. Natl. Cancer Inst. 54:1097-1101, 1975. (CA-14370)

Murad, F., Kimura, H., Hopkins, H., Looney, W.B. and Kovacs, C.J. "Increased Urinary Excretion of Cyclic Guanosine Monophosphate in Rats Bearing Morris Hepatoma 3924A," Science 190:58-60, 1975. (CA-10729)

Naseem, S.M. and Hollander, U.P. "Effects of Cyclic Adenosine 3', 5' - Monophosphate and Insulin on Plasma Cell Tumor (MPC-11) in Culture," Proc. Soc. Exp. Biol. Med. 150:352-355, 1975. (CA-14194, CA-12635, CA-05215)

Nelson, J.A. "Growth Inhibitory and Arginase Activities in Liver and Hepatoma Extracts," Proc. Soc. Exp. Biol. Med. 149:900-902, 1975. (CA-10729)

Nicolson, Garth L. "Dynamic Changes on the Surfaces of Normal and Transformed Cells and Their Relationship to the Entry of Pharmacologically Active Proteins," Am. J. Clin. Path. 63:677-684, 1975. (CA-15122)

Nicolson, Garth L. "Current Views on the Molecular Organization of Biological Membranes in Mammalian Cells," Probes and Problems (C.R. Richmond et al, eds.), USERDA Tech. Info. Ctr., Oak Ridge, Tenn., 246-253, 1975. (CA-15122)

Nicolson, G.L., Lacorbiere, M. and Eckhart, W. "Qualitative and Quantitative Interactions of Lectins with Untreated and Neuraminidase-Treated Normal, Wild-Type and Temperature-Sensitive Polyma-Transformed Fibroblasts," Biochemistry 14:172-179, 1975. (CA-15122)

Oppenheimer, S.B. "Functional Involvement of Specific Carbohydrate in Teratoma Cell Adhesion Factor," Exp. Cell Res. 92:122-126, 1975. (CA-12920)

Pederson, Thoru and Bhorjee, J.S. "A Special Class of Non-Histone Protein Tightly Complexed with Template-Inactive DNA in Chromatin," Biochemistry 14:3238-3239, 1975. (CA-12708, CA-13400)

Podolsky, D.K. and Weiser, M.M. "Galactosyltransferase Activities in Human Sera: Detection of a Cancer-Associated Isoenzyme," Biochem. Biophys. Res. Comm. 65:545-551, 1975. (CA-16703)

Roberts, J.H., Stark, P., Giri, C.P. and Smulson, M. "Cytoplasmic Poly (ADP-Ribose) Polymerase During the HeLa Cell Cycle," Arch. Biochem. Biophys. 171:305-315, 1975. (CA-13195, CA-11950)

Shepard, J.S., Wurster-Hill, D.H., Pettengill, O.S. and Sorenson, G.D. "Giesma-Banded Chromosomes of Mouse Myeloma in Relationship to Oncogenicity," Cytogenet. Cell Genet. 13:279-309, 1974. (CA-13625)

Short, J., Tsukada, K., Rudent, W.A. and Lieberman, I. "Cyclic Adenosine 3':5' Monophosphate and the Induction of Deoxyribonucleic Acid Synthesis in Liver," J. Biol. Chem. 250:3602-3606, 1975. (CA-06663)

Smoron, G.L., Kot, E., Schweppe, J.S. and Morris, H.P. "DNA and Nucleoprotein Changes in Liver and Tumor Nuclei After Irradiation," Oncology 29:320-328, 1974. (CA-10729)

Smulson, M., Stark, P., Gazzoliand, M. and Roberts, J. "Release of Template Restriction for DNA Synthesis by Poly ADP (Ribose) Polymerase During the HeLa Cell Cycle," Exp. Cell Res. 90:175-182, 1975. (CA-13195)

Steinberg, M.S. "Adhesion-Guided Multicellular Assembly: A Commentary Upon the Postulates, Real and Imagined, of the Differential Adhesion Hypothesis, with Special Attention to Computer Simulations of Cell Sorting," J. Theoret. Biol. 55:431-443, 1975. (CA-13605)

Teng, N.N.H. and Chen, L.B. "The Role of Surface Proteins in Cell Proliferation as Studied with Thrombin and Other Proteases," Proc. Natl. Acad. Sci. 72:413-417, 1975. (CA-15991)

Thrash, C.R. and Cunningham, D.D. "Growth Limitation of 3T3 Mouse Fibroblasts by Available Growth Surface Area and Medium Components," J. Cell. Physiol. 86:301-309, 1975. (CA-12396)

Wickus, G., Gruenstein, E., Robbins, P.W. and Rich, A. "Decrease in Membrane Associated Actin of Fibroblasts After Transformation by Rous Sarcoma Virus," Proc. Natl. Acad. Sci. 72:746-749, 1975. (CA-04186, CA-14051, CA-14142)

Wolf, L., Kohler, N., Roelm, C. and Lipton, A. "Initiation of DNA Synthesis in Mouse 3T3 Fibroblasts in Response to a Specific Factor," Exp. Cell Res. 92:63-69, 1975. (CA-15858)

Yurchenco, P.D. and Atkinson, P.H. "Fucosyl-Glycoprotein and Precursor Pools in HeLa Cells," Biochemistry 14:3107-3114, 1975. (CA-13402, CA-06576)

PROJECTIONS

The Tumor Biology Program will be reevaluated in the next year for its content, long-term goals, and immediate research emphases. Since this program's inception, it has been assumed that the accumulation of all fundamental knowledge of cell growth and cellular processes is important, principally because the process of tumorigenesis appears to be so complex. This is exemplified by the fact that differences are observed for nearly every cellular process that is compared between normal and tumor cells. A concerted effort will be made to determine what information is being developed or is currently available within other divisions and programs in NCI and other Institutes within NIH that can be used to arrive at the most reasonable and fruitful program approaches. These efforts will be complemented through conferences designed to explore new approaches to tumor research and to define the "most probable" cellular processes that are responsible for uncontrolled growth, loss of contact inhibition, and invasiveness. A number of specific but underdeveloped areas have been defined by the biomedical community in the last year which clearly merit future emphasis:

1. There is an increasing need to develop new approaches of experimentation with cell surface membranes. The interaction of cells and their communication with each other in the normal and abnormal states must be mediated through the cell surface membrane and it is the logical component of the cell which is directly or indirectly related to all three basic phenotypic properties of malignant tumors: loss of position control, loss of growth control, and the acquisition of invasiveness.
2. Regulation of gene expression in eukaryotes remains poorly understood and is considerably more complicated than in prokaryotes. Yet, those genes that are involved in the growth process are "turned off" when a somatic cell is differentiated but become "turned on" during tumorigenesis. Furthermore, loss of growth control and invasiveness are not necessarily genetically linked, since both benign and malignant tumors grow uncontrollably but only the malignant tumor is invasive, suggesting that both processes can be controlled separately. The process of regulation of gene expression must be understood before those genes responsible for uncontrolled growth and malignancy can be identified and controlled in the most sensible manner.
3. It is essential to develop improved in vitro and in vivo model systems that are more applicable to cancer experimentation. Considerable criticism has emerged concerning whether cells transformed in vitro are even closely representative of benign and malignant tumors in vivo. Thus, there is a clear need to demonstrate the applicability of current tissue culture cell systems to the cancerous process as well as to develop new ones. For example, model systems are needed which can provide a quantitative measure of invasiveness and tumorigenesis.

OTHER ACTIVITIES

Meetings Supported by Tumor Biology:

1. 1 R13 CA 18725-01, Fred C. Fox, "New Approaches to Research on Tumor Cell Surfaces." This conference surveyed the state of the art in mammalian cell membrane research as a basis for recommendations of new approaches aimed at

closing existing gaps in knowledge, and definition of areas of possible application to cancer prevention and therapy.

2. 1 R13 GM AM CA 22690-01, Irwin C. Gunsalus, "Tenth International Biochemistry Conference." Support of this conference represents the past general emphasis of the Tumor Biology Program to seek out all knowledge of mammalian cellular processes. Two other institutes were co-supporters.
3. 1 R13 CA 19146-01, William H. Fishman, "Conference: Gene Expression, Development and Neoplasia." This conference brought together a group of scientists, some who were conversant with model systems for the study of expression of embryonic genes in neoplastic transformation and in neoplastic cells, and others whose interests centered on the molecular mechanisms of control of gene expression in differentiation and development - the purpose being to develop profitable directions for future research in cancer.
4. 1 R13 CA 20087-01, Charles Yanofsky, "Gordon Conference on Biological Regulatory Mechanisms." Special sessions will examine and discuss recent developments of biological regulatory mechanisms in both prokaryotes and eukaryotes. The major purpose of the conference is three-fold: (1) to arrive at some unifying concepts in cellular regulation; (2) to identify problems and experimental approaches for studying regulatory phenomena and (3) to familiarize investigators of prokaryotic systems with eukaryotic systems so they can redirect their research and offer sound advice to their students.

Coordinating Activities:

Since a number of programs within DCRRC are cross-cutting in their content, efforts are being initiated to develop joint interests and planning activities in genetics and metastasis. Also contacts have been established with intramural laboratories in the Cell Biology Section of the Viral Oncology Branch of DCCP in order to maintain continuous knowledge of the state of the art and to seek advice and criticisms for program planning activities. Additional coordination of Tumor Biology Program activities should be achieved in the coming year with other intramural and contract staff as a consequence of a comprehensive "state of the art" report now in preparation.

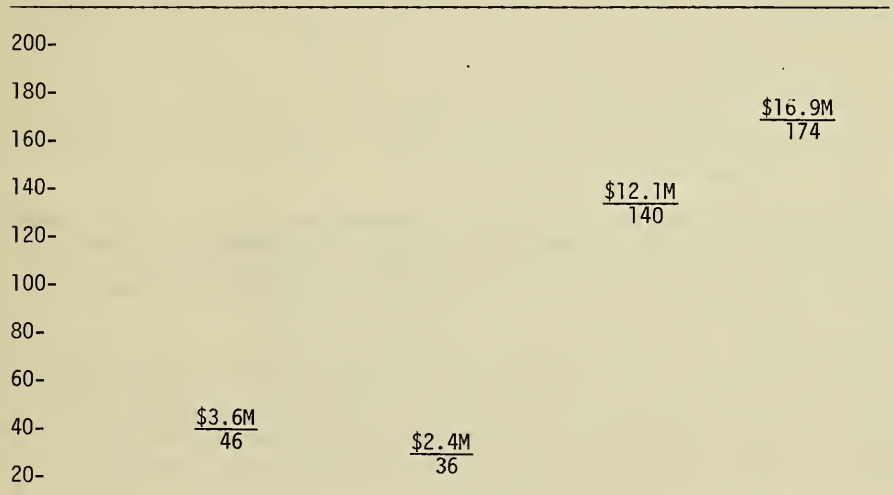
IMMUNOLOGY PROGRAM

Barbara H. Sanford, Ph.D., Program Director

HISTORY AND CONTENT OF PROGRAM

The Immunology Program has grown from 108 grants totaling $5 million in 1970 to 396 grants totaling $35.0 million at the present time, exclusive of education and training. The distribution of grants by cancer thrusts for FY 1976, was as follows:

Immunology Grants
Research and Program Projects by Thrusts



RESEARCH ACCOMPLISHMENTS

Among the published research accomplishments by grantees in the Immunology Program during 1975 were the following:

Cellular Immunology:
One of the most exciting recent developments has been the discovery of the critical role of the major histocompatibility complex antigens in target cell recognition by immune lymphocytes. Of particular interest in relation to cancer immunology has been the emerging evidence suggesting that tumor-

specific antigens may represent modified histocompatibility antigens (Germain et al., 1975).

Another rapidly developing area involves an analytical approach to cellular immunology. A relationship between cell surface antigens and functional characteristics of the cell has been demonstrated; for example, suppressor T cells have been shown to carry different Ly antigens from helper T cells (Cantor and Boyse, 1975). A different approach which allows another type of analysis of lymphocyte subpopulations is provided by recently developed methods for separating antigen-specific lymphocytes (Heber-Katz and Wilson, 1975). Taken together, these advances give great promise for an increased understanding of the lymphocyte in the near future.

Antibody Studies:
Studies of pathological IgA immunoglobulins continue to provide information pertinent to the general question of neoplastic transformation of normal antibody-mediated immunity into malignant plasmacytoma. Patients have been found who produce defective immunoglobulin molecules, with each patient carrying his own unique sequence. These differences in immunoglobulin structure indicate that although the patients may have similar clinical symptoms the transformation into a tumor cell has occurred at different sites, although always at the level of genetic control of immunoglobulin synthesis (Putnam, 1975).

Evidence was obtained for a new class of immunoglobulin (IgD) which appears to be the major surface immunoglobulin of murine B lymphocytes. The heavy chain of IgD was found to have a molecular weight of 65,000 and was shown to be antigenically distinct from other mouse immunoglobulins (Vitetta et al., 1975).

Modulation of the immune response by anti-idiotype sera has also recently generated much interest. The observation that a specific immune response can be stimulated or suppressed even in the absence of the relevant antigen has provoked interest in investigating the basic mechanisms involved and in studying the possible application of this type of manipulation to cancer and transplantation (Sakato and Eisen, 1975).

Role of the Immune State in Carcinogenesis:
The hypothesis that immune surveillance serves to protect individuals against development of tumors has continued to come under severe attack (Prehn, 1975; Stutman, 1975; Outzen et al., 1975). A substantial body of data has been developed which indicates that immunosuppressed hosts do not generally demonstrate an overall increase in tumor development. There is, in fact, some recent evidence suggesting that under certain conditions an immune response may stimulate the development of tumors. The role of the immune system in carcinogenesis is still not clear, however, and there is promise of controversy in this area for some time to come.

Another type of ongoing study of immune status is concerned with the effect of oncogenic virus infection on immune responses. Phagocytic and migratory functions of peritoneal macrophages from Friend virus-infected mice have

been shown to be significantly depressed as compared with normal controls. Leukemic macrophages exposed in vivo and in vitro to statolon regained normal function (Levy and Wheelock, 1975).

Immunotherapy:
The UCLA protocol to investigate immunotherapy for malignant melanoma has been continued, with BCG or BCG plus tumor vaccine used following surgery to remove gross disease. Results so far suggest that patients with regional metastases given either type of immunotherapy have fewer recurrences than those treated with surgery alone, but the number of patients treated to date is small and differences are not yet statistically significant (Morton et al., 1975).

Follow-up studies on antitumor effects of cell-mediated reactions to superficial epidermal tumors have been continued in patients with skin cancers. In two successive groups of patients with multiple epitheliomatoses 60 to 90% of tumors were eradicated, with no recurrences during observation periods of 5 to 10 years (Klein et al., 1975).

REFERENCES

Cantor, H. and Boyse, E.A. "Functional Subclasses of T Lymphocytes Bearing Different Ly Antigens," J. Exp. Med. 141:1376-1389, 1975. (CA-16889)

Germain, R.N., Dorf, M.E. and Benacerraf, B. "Inhibition of T Lymphocytes Mediated Tumor Specific Lysis by Alloantisera Directed Against the H-2 Serological Specificities of the Tumor," J. Exp. Med. 142:1023-1028, 1975. (CA-14723)

Heber-Katz, E. and Wilson, D. "Collaboration of Allogeneic T and B Lymphocytes in the Primary AB Response to Sheep Erythrocytes In Vitro," J. Exp. Med. 142:928-935, 1975. (CA-15822)

Klein, E., Holterman, O.A., Helm, F., Rosner, D., Milgrom, H., Adler, S., Stoll, H.L. Jr., Case, R.W., Prior, R.L. and Murphy, J.P. "Immunologic Approaches to the Management of Primary and Secondary Tumors Involving the Skin and Soft Tissues." Review of a ten year program. Transplantation Proceedings 7:297-315, 1975. (CA-13599)

Levy, M.H. and Wheelock, E.F., "Impaired Macrophage Function in Friend Virus Leukemia; Restoration by Statolon," J. Immunology 114:962-965, 1975. (CA-12461)

Morton, D.L., Golut, S.H., Sulit, H.L., Gupta, R.K., Eilber, F.R., Holmes, E.C. and Sparks, F.C. "Immunologic and Clinical Responses to Active Immunotherapy of Malignant Melanoma." In Fundamental Aspects of Neoplasia, A. Gottlieb, O.J. Plescia and D.H.L. Bishop (eds.), Springer-Verlag, N.Y., pp. 181-201, 1975. (CA-12582)

Outzen, H.C., Custer, R.P., Easton, G.J. and Prehn, R.T. "Spontaneous and Induced Tumor Incidence in Germfree 'Nude' Mice," J. Reticuloendothelial Society 17:1-9, 1975. (CA-08856)

Prehn, R.T. "Does Immunity Promote or Inhibit Tumor Growth?" In: Viruses and Immunity, Acad. Press N.Y., 1975, pp. 59-66. (CA-08856)

Putnam, F.W. in The Plasma Proteins, Vol. 2, Ed. F.W. Putnam, Academic Press, N.Y., 1975. (CA-18497)

Sakato, N. and Eisen, H.N. "Antibodies to Idiotypes of Isologous Immunoglobulins," J. Exp. Med. 141:1411-1426, 1975. (CA-15472)
Stutman, O. "Tumor Development After Polyoma Infection in Athymic Nude Mice," J. Immunol. 114:1213-1217, 1975. (CA-17818, CA-15988)
Vitetta, E.S., Melcher, U., McWilliams, M., M.E., Phillips-Quagliata, J.M. and Uhr, J.W. "Cell Surface Immunoglobulin XI. The Appearance of an IgD Like Molecule on Murine Lymphoid Cells During Ontogeny," J. Exp. Med. 142:206, 1975.(CA-08627)

PROJECTIONS

Basic Immunology:
The current level of scientific interest in such problems as the role of the major histocompatibility locus in immune reactions is very high and scarcely requires additional stimulation by NCI. On the other hand, adequate support is urgently needed and it is, therefore, important that the DCRRC Immunology Program continue an active effort to support basic immunobiology. Support of workshops and other types of informational exchange on topics in rapidly advancing areas of immunobiology is also justified.

There are other areas of immunobiology, however, where stimulation of more work is indicated. These include studies of immunogenicity of spontaneous tumors, investigations of the mechanisms involved in spontaneous killing of human target cells by lymphocytes, and studies of genetic factors affecting immune responses other than those related to the major histocompatibility locus.

Identification and Characterization of Human Tumor-Associated Antigens:
Although a large number of human tumor-associated antigens have been reported, it is not clear whether the antigenic specificities being detected are related to individual or cross-reacting tumor antigens or to fetal, histocompatibility, or autoimmune antigens. Identification and characterization of new human tumor antigens require a combination of clinical, immunological, and biochemical expertise. Since a multidisciplinary team focusing on this problem appears to be the most promising approach, program projects in this area are being encouraged.

New Approaches to Immunotherapy:
Although some success is being achieved with the immunotherapeutic approaches now in use, most investigators involved in clinical immunotherapy agree that there is a need for careful model studies on new approaches, preferably those that stress mechanisms of action rather than simply the testing of new agents according to various schedules. Investigators are being encouraged to submit grant applications for projects of this type.

One type of immunotherapy in which there has been a recent resurgence of interest is the use of antibodies to deliver radiation or drugs to tumors. Since a number of laboratories throughout the world are now involved in this type of effort, a workshop or conference in this area might be useful and is under consideration.

OTHER ACTIVITIES

Efforts at attracting able young investigators into immunology receive high priority in the Immunology Program. Small regional meetings between young investigators and leaders in the field are being tried on a pilot basis as a means of stimulating interest in tumor immunology. The first such meeting was held in Bar Harbor, Maine, in September 1975 and was enthusiastically received by young investigators. A second meeting for investigators from the southeast was held in Stone Mountain, Georgia, in February 1976; again results indicated that the meeting succeeded in increasing the interest of young investigators in tumor immunology.

Close coordination is maintained with intramural and contract staff. The Program Director attends meetings with contract committees when program planning is involved and is in frequent contact on other matters of mutual interest.

A joint meeting for investigators with NCI grants or contracts in immunobiology has been planned by the DCBD Associate Director for Immunology and the DCRRC Program Director for Immunology. Over 200 investigators are expected to attend this meeting at Hilton Head, South Carolina in June, 1976.

Consultants:
Among those providing advice to the Immunology Program Director on an ad hoc basis were: Dr. D. Bernard Amos, Dr. Baruj Benacerraf, Dr. Barry Bloom, Dr. Ingegerd Hellstrom, Dr. Evan Hersh, Dr. Hilary Koprowski, Dr. Donald Morton, Dr. Richmond Prehn, Dr. David Pressman, Dr. George Santos, Dr. Stewart Sell, Dr. Osias Stutman, Dr. Charles Todd, Dr. Darcy Wilson, and Dr. Henry Winn.

CARCINOGENESIS PROGRAM

Thaddeus J. Domanski, Ph.D., Program Director

HISTORY AND CONTENT OF PROGRAM

The present Carcinogenesis Program, initiated during FY 68, is devoted to the development of facts and insight essential to the prevention of human cancer, caused or promoted by chemical or physical agents. Programmatic focus is on environmental carcinogenesis, but with a similar amount of support accorded to more fundamental studies, consisting principally of cancer biochemistry and cancer endocrinology.

Traditional grants assigned to the Carcinogenesis Program are grouped within one of two categories respectively referred to as Chemical Carcinogenesis and Biochemistry of Cancer. Within the Chemical Carcinogenesis category the emphasis is on the action, mechanism of action, and physiological disposition of chemical carcinogens. Within the Biochemistry of Cancer category the focus is on the characteristic biochemistry of the cancer cell and on biochemical comparisons between normal and tumor tissues. In terms of the National Cancer Program classification scheme, Chemical Carcinogenesis grants are assigned to Cause and Prevention (Thrust 1), whereas Cancer Biochemistry grants are assigned to Cancer Biology (Thrust 4). In FY 76 the Chemical Carcinogenesis category consisted of 153 grants, supported at a cost of approximately $8.7 million, while the Cancer Biochemistry category consisted of 137 grants, costing approximately $8.1 million.

Other than traditional grants, the Carcinogenesis Program supported 17 Program Projects, and one Exploratory Grant. In terms of major effort, ten of these classify as Cause and Prevention (Thrust 1) projects; the remaining seven as Cancer Biology (Thrust 4). The cost of the Thrust 1 projects was approximately $9.5 million; that of the Thrust 4 projects, approximately $3.5 million. An additional 32 traditional grants and two program projects received no FY 76 funds, but received full administrative support for at least part of the year.

RESEARCH ACCOMPLISHMENTS

Published research accomplishments from selected Carcinogenesis Program grants follow:

Because of the current belief that metabolic intermediates of aromatic amines are further activated to electrophilic reactants capable of binding covalently to cellular nucleophiles, and because esters of the strongly carcinogenic N-hydroxy-2-fluorenylacetamide (N-OH-2-AAF) give rise to an electrophilic reactant, the acetate esters of N-OH-3-AAF and N-OH-4-AAF were prepared and tested in rats (Yost et al., 1975). The order of carcinogenicity of the isomeric esters followed that of the parent hydroxamic acid: N-acetoxy-2-AAF>N-acetoxy-3-AAF>>N-acetoxy-4-AAF. These results

are additional evidence that the structure (specifically, the point of attachment of the nitrogen atom on the aromatic moiety) is a major determinant of the carcinogenicity of these compounds. The weak electrophilicity of N-acetoxy-4-AAF is consistent with its marginal carcinogenicity. This implies the existence of a threshold for interaction of the carcinogen with the nucleophilic target, which must be exceeded if a compound is to display cancer-inducing activity. Interaction of the 4-isomer with noncritical nucleophiles such as chloride, acetate, and water may account for its relative lack of carcinogenic activity. N-acetoxy-3-AAF, by its relative lack of reactivity toward nucleophiles, seemingly contradicted the concept linking such reactivity with carcinogenesis. There are, however, two alternate pathways, not involving an N-acyl-N-arylnitrenium ion but yielding electrophilic species, which are proposed to account for the carcinogenicity of the 3-isomer. One of these has been tested (Yost et al., 1975) and the results indicate that N-acetoxy-3-AAF is an even better acetyl donor than N-acetoxy-2-AAF; rat liver RNase is extensively modified by reaction with the 3-isomer.

Human tissues are capable of effecting the initial metabolic transformation believed to be required for activation of aromatic amines. They have now been shown also to possess an enzyme capable of promoting the introduction of the arylamine moiety of N-OH-2-AAF and N-OH-N-4-biphenylacetamide (N-OH-BPAA) into nucleic acids (King et al., 1975). Incubation of N-OH-2-AAF or N-OH-BPAA and tRNA led to formation of arylamine-substituted nucleic acid adducts in supernatants of human small intestine, liver, or colon homogenates. The function of acyltransferase in tumor induction by arylamines is at present unknown. No direct correlation is discernable between acyltransferase activity and carcinogenic response to aromatic amines (King and Olive, 1975), but the chemical induction of tumors is probably influenced by multiple factors whose interrelationships will have to be clarified before it is possible to establish the function of acyltransferase or other activation mechanisms in the carcinogenic process.

Extension of the aromatic amine data to the related azo dyes has been hindered by an inability to synthesize the presumed N-hydroxy metabolite of the prototype azo dye N-methyl-4-aminoazobenzene (MAB). Release of 5-methylmercapto-MAB and of N-(guan-8-yl)-MAB derivatives from the liver protein and nucleic acids of rats given MAB provided circumstantial evidence for the formation of an ester of N-OH-MAB or a similarly reactive metabolite *in vivo*, suggesting a similar mechanism of activation for azo dyes and aromatic amines (Lin et al., 1975b). N-benzoyloxy-MAB, a model of reactive esters which might be formed *in vivo* from N-OH-MAB, has been synthesized and tested in rats, where it induced sarcomas at the injection site (Lin et al., 1975a). Analysis of the reactions of the synthetic ester and of MAB shows that they are similar to the reactions of AAF, its N-OH metabolite, and synthetic esters of N-OH-AAF. Each type of ester reacts *in vitro* at neutrality with RNA, DNA, and guanine-containing nucleosides or nucleotides to form derivatives in which the amino nitrogen of MAB or the amide nitrogen of AAF is attached to carbon 8 of the guanine residues. Similarly, MAB, AAF, and N-OH-AAF are metabolized in rat liver *in vivo* to rRNA- and DNA-bound derivatives in which the amino nitrogen of MAB or the amide or amino nitrogens of AAF and AF, respectively, are attached to carbon 8 of guanine

bases in the nucleic acids (Lin et al., 1975a).

The N-benzoyloxy derivatives of MAB, its 4'-methyl and 4'-ethyl derivatives, and N-ethyl-4-aminoazobenzene were compared for carcinogenicity and reactivity with nucleophilic reagents (Wislocki et al., 1975). Each of the four esters had a similar degree of nonenzymatic reactivity with methionine and guanosine at neutral pH. Each of the dyes induced sarcomas at the site of subcutaneous injection in rats, but N-benzoyloxy-MAB was considerably more oncogenic than the others, possibly because of its greater stability in neutral lipid. Neither the electrophilic reactivities nor the subcutaneous tumor inducing abilities of these compounds paralleled the hepatocarcinogenic activities of the parent dyes. While these data may indicate that the N-benzoyloxy dyes are not ultimate carcinogens, the lack of correlation between carcinogenicity and reactivity with nucleophiles may be otherwise explained. For example, the limiting factor in carcinogenicity of the N-benzoyloxy dyes may be their stability at the subcutaneous site from the time of injection until removal from the depot site. It is also possible that reactions with nucleophilic sites in macromolecules would correlate better with carcinogenicity than would reactions with small molecules, especially if the ability of the dye to associate physically with nucleic acids or proteins facilitates their reaction. Alternatively, the critical reactions for carcinogenesis may be quantitatively minor ones for which the extent of reaction does not parallel that of the major reactions with methionine and guanosine.

One approach to determining the relevance of the various effects of chemical carcinogens is to compare the early biochemical effects of a carcinogen with those of a closely related noncarcinogenic compound. This type of study was recently conducted in relation to hepatic carcinogenesis in the rat (Yager and Potter, 1975). Two hepatocarcinogens, AAF and 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) and the relatively noncarcinogenic 2-methyl-4-dimethylaminoazobenzene (2-Me-DAB) were compared for their effects on rat liver DNA stability and new DNA synthesis. Rat liver nuclear DNA is relatively stable; rapid metabolic turnover does not occur in the absence of toxic influences and loss of prelabeled DNA may reflect cell death. 3'-Me-DAB caused a dose-dependent loss of prelabeled DNA, demonstrating the cytoxicity of this carcinogen. 2-Me-DAB caused only a small loss of prelabeled DNA, and AAF, despite its potent hepatocarcinogenicity, caused none.

Experiments designed to separate liver regenerative hyperplasia from premalignant hyperplasia revealed both an early and a delayed elevation of thymidine incorporation into liver DNA of rats fed 3'-Me-DAB (Yager and Potter, 1975). Feeding of 2-Me-DAB depressed thymidine incorporation. Feeding isomolar amounts of 3'-Me-DAB and AAF caused comparable early increases in thymidine incorporation, followed by a rapid return of incorporation to control levels upon removal of the carcinogens from the diet. The initial hyperplastic response caused by 3'-Me-DAB and AAF may not have the same cause. With the former, hyperplasia may represent regeneration in response to its cytotoxic effects whereas in the case of AAF, which appears much less cytotoxic, the comparable degree of hyperplasia may result from a direct stimulation of cell proliferation.

Apparently a complete hepatocarcinogen must damage the cell and cause a hyperplastic response; if it can accomplish the former but not the latter, exogenous influences must act to continue the carcinogenic process. This two-step process has been well documented in skin carcinogenesis where some chemicals have been found to be tumor initiators, others tumor promoters, and still others complete carcinogens. Initiation is generally regarded as an irreversible process which can be produced by sufficient treatment with a subcarcinogenic dose of a physical or chemical carcinogen, while promotion is a reversible process requiring repeated application of a promoting agent which stimulates the initiated cells.

Inhibition of RNA synthesis is a common effect of the acute action of several hepatocarcinogens. With the possible exception of aflatoxin, this inhibition does not seem to be accompanied by an inactivation of the template properties of chromatin (Herzog et al., 1975). The decreased nuclear RNA synthesis observed in rat liver following administration of N-hydroxy-AAF (an intermediate in the activation of AAF) can be accounted for entirely by an inhibition of RNA polymerase activity; both nucleolar and nucleoplasmic polymerases are affected. The presumed basis for carcinogenicity by this compound is arylamidation of DNA, RNA, and/or protein. It has not been determined whether this mechanism is responsible for inhibition of the RNA polymerases. There is reason to suspect, however, that it is this interaction of N-OH-AAF with nuclear proteins directly or indirectly that interferes with the function of the RNA polymerases. Binding of N-OH-AAF to histones and acidic proteins of rat liver nuclei has been reported by several groups.

At least two possibilities can be suggested for the potential significance of RNA polymerase inhibition with respect to the mechanisms of information generation during carcinogenesis (Herzog et al., 1975). The inhibited polymerases could have an altered template specificity allowing for transcription from previously repressed genes. On the other hand, inhibition of the polymerase function without an apparent effect, at least quantitatively, on the chromatin template would reduce the level of genetic transcription without interfering with the integrity of this information. This could result in fewer repressors being made and in some genes being derepressed.

Chemical carcinogens show varying degrees of tissue specificity or, rather, tissue characteristics confer varying degrees of susceptibility to chemical carcinogens. The distribution of activating enzymes appears to be a determinant in the case of the nitrosamines (Swenberg et al., 1975). In contrast, the closely related nitrosamides such as methylnitrosourea (MNU) are believed to be activated nonenzymatically and so do not derive tissue specificity via this pathway. Nevertheless, when administered intravenously at optimal dose schedules, MNU and related nitrosamides exhibit a pronounced neurospecificity. The conditions influencing the specificity of these agents have been investigated (Swenberg et al., 1975) and the results suggest several possible explanations for the disproportionate sensitivity of neural tissues. MNU, because of its basic urea structure, may penetrate the blood-brain barrier more readily than some other carcinogens. Another possibility is that DNA damage caused by MNU is repaired less efficiently in the nervous system than in other tissues.

The ability of a cell to repair its DNA is a fundamental defense mechanism against agents which destroy the integrity of this vital macromolecule. The possibility has been examined that premalignant hyperplastic liver nodule cells have defective DNA repair mechanisms which make them more susceptible to neoplastic transformation. The level of unscheduled DNA synthesis (UDS, a parameter of DNA repair) in the parenchymal cells from hyperplastic nodules and from the entire liver of rats fed N-2-fluorenyl-acetamide was studied and compared with that of normal liver cells (Kitagawa et al., 1975). Tritiated thymidine labeling was variable among individual nodules and animals and fluctuated as a function of the number of days in culture. In general, however, the level of UDS in the cells from hyperplastic nodules was always higher than, or similar to, that of normal liver cells. Thus, although AAF induces considerable DNA damage, the nodule cells are not more readily transformed as a result of lowered DNA repair capacity.

The ability of an active metabolite of AAF, N-acetoxy-AAF, to induce mutations to azaguanine resistance was quantitatively investigated in diploid human cells and shown to be dose-dependent (Maher and Wessel, 1975). The azaguanine resistance was shown to be heritable in the absence of mutagen or selective agent and the cells of the mutant clones were shown to retain normal sensitivity to N-acetoxy-AAF. These findings strengthen the correlation which has been observed between carcinogenicity and mutagenicity and support the concept of somatic mutation as a carcinogenic mechanism. The presence on the DNA of the stably bound, bulky aromatic residue may be directly or indirectly related to the mutagenic effect of N-acetoxy-AAF. Inaccuracies in the repair process itself may play a role in the production of mutations.

4-Nitroquinoline 1-oxide (4NQO), a compound which is highly mutagenic in bacteria, induces repairable damage in mammalian DNA. Subcutaneous injection of 4NQO induces pulmonary tumors in rats. With the availability of a method to study DNA damage and repair in the intact animal, the tissue specificity of the damage and repair induced in the rat by 4NQO was examined (Cox and Irving, 1975). DNA damage was detected in the liver, lung, and kidney, with the lung showing the greatest amount of damage. The preferential damage of rat lung DNA by 4NQO correlates with the specificity of this carcinogen to induce pulmonary tumors. All three tissues were able to repair the damaged DNA within hours, in contrast to the situation with a number of alkylating carcinogens, where DNA is not completely repaired even after two weeks. 4NQO may bind covalently or intercalate in DNA to produce a distortion in the molecule that is acted upon immediately by nucleases, thus inducing rapid repair of damage.

It is conceivable that, in the absence of efficient DNA repair, a deletion or rearrangement could result in certain critical segments in the genome of a mammalian somatic cell being eliminated or altered in such a way as to produce malignancy. The frequency of mutations per dose of "K-region" epoxides of several hydrocarbons has been shown to be significantly higher than normal in excision repair-deficient xeroderma pigmentosum (XP) cells (Maher and McCormick, 1975a). However, at equicytotoxic doses (i.e., doses which result in an equal percent survival), the frequency of mutations in-

duced by the hydrocarbons in an XP strain which carries out little or no excision repair was essentially equal to that measured in normal cells which had carried out extensive excision repair of their DNA. These results provide evidence that the repair processes in human cells do not themselves introduce the mutations. They support the hypothesis that the cytotoxic and mutagenic effects of exposure to the hydrocarbons result from unexcised DNA lesions.

In bacteria, excision repair has been shown to be essentially error-free; mutations arise as a result of unexcised damage. The results cited above indicate that the same is true of humans. Even though XP patients generally have lower than normal rates of excision repair, their excision repair is qualitatively normal. Results of studies with aromatic amide derivatives and ultraviolet radiation, like those with the hydrocarbon epoxides, support this conclusion (Maher and McCormick, 1975a,b). Thus, the induced mutation frequencies of the two excision repair-deficient XP strains and of a strain of normal cells, expressed as a function of the percent survival of the respective strains, are equal.

Cells from a few XP patients have normal rates of excision repair, but are deficient in postreplication repair. One of these variant strains, XP4BE, was used to compare the effects of excision and postreplication repair on the cytotoxicity and mutagenicity of UV radiation (Maher et al., 1975). As in previous studies, the doses of UV which result in 37% survival in the excision repair-deficient strains were the same doses required to induce mutations at a frequency of 10 per 10^5 survivors. In contrast, XP4BE had a lower rate of survival than normal cells and had a much higher frequency of mutations per survivor. These results demonstrate the importance of postreplication repair in protecting rapidly dividing cells from mutagenic lesions.

The effects of two potent tumor promoters on two DNA repair processes have recently been examined in human amnion (AV_3) cells (Trosko et al., 1975). Cells were treated with low initiating doses of either UV radiation or N-acetoxy-AAF. Excision repair (as measured by UDS) was then monitored in the presence or absence of nontoxic levels of either 12-O-tetradecanoyl-phorbol-13-acetate (TPA), phorbol-12,13-dibenzoate (PDB), both potent tumor promoters, or phorbol, a nonpromoter. Neither promoter inhibited DNA repair synthesis occurring in response to low doses of the carcinogens. In addition, TPA did not inhibit postreplication repair in UV-irradiated Chinese hamster cells. Previous studies, on which the tested hypothesis was based, either used high UV doses, where cell survival was low, or did not report the dose used. DNA repair is biologically significant only if it occurs in a cell which will survive and in which the effects of repair can be observed. It is obvious that promotion can occur only in surviving cells. In addition, the hypothesis itself is insufficient to account for promotion (Trosko et al., 1975). Promotion can occur weeks or even months after carcinogen treatment, whereas repair of most of the repairable DNA damage is completed within hours, at least in vitro. However, since promoters stimulate DNA synthesis and cell division, their application prior to the complete removal of unrepaired lesions will cause the cell to use

its error-prone postreplication repair process to fill gaps in newly synthesized DNA which are adjacent to the unrepaired lesions. This error-prone process may result in somatic cell mutations leading to either no adverse effect on the cell, to death, or to neoplasia.

The sequence of biochemical events in mouse epidermis after a single application of a tumor promoter resembles that seen in other tissues undergoing rapid proliferation following appropriate stimuli: augmentation of RNA, protein, and DNA synthesis, and of epidermal histone phosphorylation. In addition, L-ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase appear to play prominent roles in eukaryotic tissues undergoing rapid proliferation. These enzymes and their products, putrescine and the polyamines spermidine and spermine, are greatly increased in tissues undergoing physiological or pathological proliferation. The kinetics of the activities of the two enzymes have been examined in mouse skin after application of tumor promoters (O'Brien et al., 1975a). A single topical application of croton oil or 12-O-tetradecanoylphorbol-13-acetate (TPA) resulted in a rapid, transient stimulation of both enzyme activities which appeared to be due to increased de novo synthesis. The data suggested that, within two hours after a single application of an active promoter, and before the general increase in total RNA and protein synthesis, synthesis of specific mRNAs for the two enzymes occurs. This is one of the earliest specific biosynthetic events known to take place after promoter treatment and it may be necessary for the subsequent increase in DNA synthesis and cell division.

Further investigation of enzyme induction by tumor promoters revealed that mouse skin tumors produced by the two-stage procedure had consistently high levels of ornithine decarboxylase activity but variable levels of S-adenosyl-methionine decarboxylase activity (O'Brien et al., 1975b). Substances which induce hyperplasia in mouse skin, but which do not function as promoters, caused no increase in ornithine decarboxylase activity although they increased S-adenosyl-methionine decarboxylase activity to the same extent as promoters (O'Brien et al., 1975b). All promoters tested, although of diverse chemicobiological properties, were effective inducers of ornithine decarboxylase, while no nonpromoters had this effect. Within a series of structurally related promoters (phorbol esters), promoting ability correlated well with the magnitude of the ornithine decarboxylase response.

A similar lack of promoting activity on the part of a reactive carcinogen metabolite was seen in a study of hepatic nodules induced in the rat by subcarcinogenic doses of AAF (Becker, 1975). Subsequent administration of a subcarcinogenic dose of the N-hydroxy metabolite of AAF failed to complete the process of carcinogenesis in the nodules induced by AAF; most of the nodules regressed. Substitution of a subcarcinogenic dose of dimethylnitrosamine for N-hydroxy-AAF resulted in hepatocellular carcinoma development in 100% of the animals previously treated with AAF. The yield of hepatomas in this study far exceeded that reported to occur when dimethylnitrosamine is administered during liver regeneration; the lag time was much shorter, and the dose was below that reported to be effective. These results support the concept that the nodules produced by subcarcinogenic ingestion of

AAF are not composed simply of normal hepatocytes undergoing compensatory regeneration, but rather consist of cells that have been altered by the carcinogen so that they are hyper-susceptible to further carcinogenic evolution. The question of whether the alteration induced by the initiating carcinogen is irreversible is not resolved by this study. True initiation, as seen in the mouse skin system, has generally been regarded as irreversible.

The impaired response of hepatoma enzymes to hormonal or substrate regulation has been well documented. Studies of enzyme regulation in liver undergoing malignant conversion have, on the other hand, produced conflicting results, possibly because of the tendency to treat the premaligant liver tissue as a homogeneous mass of cells. To overcome this possible source of error, a recent study (Kitagawa and Pitot, 1975) employed both enzyme histochemistry and autoradiography to study changes in the response of two rat liver enzymes to dietary manipulation during carcinogen exposure. Serine dehydratase, diffused throughout the cytoplasm of hepatocytes in the periportal zone of untreated liver, was markedly induced during feeding of diethylnitrosamine (DEN) but only slightly induced during feeding of AAF. The enzyme was deficient and not inducible in hyperplastic nodules early in their development but, subsequently, both the levels of the enzyme and its inducibility increased in the majority of the nodules. In general, similar changes were seen in glucose-6-phosphatase activity and inducibility but there was a minor group of nodules in which deficiencies of both these enzymes persisted and in which enzyme induction was not observed.

Whereas there is considerable evidence to support the concept that malignant transformation involves a process of "dedifferentiation," these results demonstrate that even tissue which has undergone what appears to be an essential step in the process of hepatic tumor development can exhibit maturation in its response to hormonal regulation as well as in its endogenous enzyme levels after prolonged carcinogen feeding.

Analysis of hormone-dependent rat mammary adenocarcinomas has clearly identified estrogens and prolactin as the two major hormone types responsible for regulating the growth of these tumors. It appears that prolactin is the prime stimulus for breast tumor growth but that estrogen-mediated events are necessary for prolactin to exert its influence. The MTW9-MA rat mammary tumor is markedly deficient in both estrogen and prolactin binding sites (Costlow et al., 1975). Its autonomy may thus be explained by a failure of circulating hormones to recognize the tumor cells as targets. The coordinate reduction in binding sites for estrogens and for prolactin in MTW9-MA suggested that loss of both types of receptor might be a general feature of the transition to independent tumor growth.

In vivo studies of R3230AC have shown (Cohen and Hilf, 1975) that this tumor also responds to insulin. The tumor grew in diabetic rats at a rate equal to or greater than that in intact rats but tumor growth was reduced when daily doses of insulin were administered. Moreover, treatment with estrogen inhibited tumor growth in diabetic or intact rats simultaneously treated with insulin; the effects of the two hormones ap-

peared to be additive. The R3230AC tumor may be an experimental analog of those recurrent human breast cancers which arise after ablation of steroid hormone-producing organs. The characteristics of such tumors may be quite different from those of the primary lesion, which may be hormone-dependent.

Some of the findings derived from studies of hormone receptors in rat mammary tumors are already being applied to the human disease. The current status of estrogen receptors in human breast cancer has recently been reviewed (McGuire, 1975). Data on 436 clinical trials contributed from a dozen centers throughout the world now clearly indicate that if a patient's tumor does not contain estrogen receptor, there is virtually no chance of inducing tumor regression by endocrine therapy. Patients can thus be spared unrewarding endocrine ablative therapy if estrogen receptor assays are performed routinely. The prognostic significance of the receptor values for response to endocrine therapy does not seem to depend on the type of tumor tissue that is biopsied for the assay: values from primary or metastatic tumors predict equally well.

Of the patients whose tumors do contain estrogen receptors, only 55 to 60% respond to endocrine therapy (McGuire, 1975). Since binding to receptors is an early step in hormone action, it is possible that in receptor-positive tumors where endocrine manipulations fail, the lesion is at a later step. If so, an ideal marker of an endocrine-responsive tumor would be a measurable product of hormone action rather than the initial binding step. Because in estrogen target tissues the synthesis of progesterone receptors depends on the action of estrogen, progesterone receptors (PgR) were suggested as such a marker (Horwitz et al., 1975). The presence of PgR in tumors containing estrogen receptors (ER) would indicate that the tumor is capable of synthesizing at least one end product under estrogen regulation. It was predicted that breast tumors with PgR would respond to hormonal therapy but that tumors with ER but not PgR would be resistant to endocrine therapy. In a preliminary survey, PgR was detected in 56% of tumors with ER but, as expected, in no tumors without ER. This approximates the percentage of ER-positive patients who respond to endocrine therapy, so that this result is consistent with the hypothesis that PgR is a marker of hormone-responsive breast tumors. Confirmation of the hypothesis requires direct correlation of the presence of PgR with objectively defined clinical remission. Only nine patients have been completely evaluated to date but the findings support the hypothesis: the only remissions among these nine patients occurred in the three patients with ER-positive, PgR-positive tumors. No remissions occurred in the four ER-negative, PgR-negative patients or in the two ER-positive, PgR-negative patients.

Deletion of normal tissue antigens and acquisition of new antigens have both been observed in a number of human tumors. The suggestion that some of these new antigens represent incompletely synthesized normal antigens would account for the simultaneous deficiency of the latter. To determine whether reduction of normal tissue antigens on tumor cells might be related to a deficiency in glycoprotein glycosyltransferase activity, several of these enzymes were measured in homogenates of normal and

neoplastic human colon epithelium (LaMont and Isselbacher, 1975). These enzymes transfer monosaccharides either to partially completed glycoproteins on the cell membrane (endogenous acceptors) or to added glycoprotein or monosaccharide substrates (exogenous acceptors). The levels of exogenous galactosyltransferase and fucosyltransferase and endogenous N-acetylglucosaminyltransferase were higher in the normal tissue. The levels of exogenous and endogenous sialyltransferase and of endogenous galactosyltransferase and fucosyltransferase were comparable in homogenates of normal or cancer cells. That the appearance of carcinoembryonic antigen (CEA) in colon cancer may be the result of glycosyltransferase deficiency is supported by the finding in this study that galactosyltransferase and fucosyltransferase from normal colon epithelium are more active with CEA as substrate than are the corresponding transferases from cancerous colon tissue.

The enzyme abnormalities observed in tumors might result from a disturbance in almost any one of the many complex steps involved in the transcription and translation of genes into their final products. The fact that many tumor enzymes resemble embryonic forms suggests that defective gene regulation rather than a structural gene mutation is involved. Putative regulatory molecules which may be useful in tumor detection have been identified in the peripheral blood plasma of rats bearing either transplanted hepatomas or autochthonous chemically induced mammary tumors (Schumm and Webb, 1975). The components were assayed by measuring their ability to stimulate mRNA release from isolated nuclei in a cell-free system of rat liver nuclei in fortified homologous cytosol. In crossover experiments with nuclei and cytosol from different tissue sources, non-dialyzable components regulating nuclear processing and/or nucleoplasmic transport of mRNA *in vitro* showed significant qualitative differences in normal, regenerating, and neoplastic liver. The components are inferred to be involved in nucleocytoplasmic controls at the post-transcriptional nuclear level *in vivo*. Qualitative changes in cytosol regulatory molecules, such as those seen in these studies, may account for the apparent loss of nuclear restriction in the cancerous or precancerous cell; i.e., much of the mRNA retained or destroyed in the nucleus of normal cells appears in the cytoplasm of cancer cells.

Liver cytosol from tumor-bearing hosts behaved abnormally in these studies when compared with that derived from normal liver in a cell-free system containing prelabeled liver nuclei. Substitution of tumor or host liver cytosol for normal liver cytosol resulted in: (1) slightly reduced RNA transport; (2) a change in the size distribution of the released RNA; (3) a change in the sequence homology of the transported RNA; and (4) abnormal amounts of poly A-containing mRNA being released to the cytosol. The test system derived from rat liver permits monitoring plasma from other species, and the regulatory components in the plasma appear also to be species-nonspecific. The high concentrations of apparently qualitatively different regulatory macromolecules in the plasma of tumor-bearing animals suggest that the nuclear restriction assay could be used as a cancer diagnostic test. The present assay system, which may not be completely optimized, seems to be of equal or greater sensitivity compared with the CEA immunoassay which detects gastrointestinal and related types of cancer (Schumm and Webb, 1975).

An apparently new antigen, tentatively called PN (preneoplastic), has been detected in hyperplastic nodules of rat liver after treatment of the animals with hepatocarcinogens (Okita et al., 1975). It has been found in every early and late hyperplastic nodule and in every primary hepatoma induced by any one of five carcinogens in three strains of rats. Thus far, it has not been detected in normal adult rat liver, liver surrounding hyperplastic nodules or malignant tumors, fetal liver, amniotic fluid, normal adult rat serum, sera from rats with hyperplastic nodules or primary hepatomas, or various normal rat tissues. The possibility that the PN antigen is a liver protein modified by interaction with a chemical carcinogen is rendered unlikely by its presence in hepatocytes altered by one of several chemically unrelated compounds, and by its presence in hepatomas transplanted up to 150 times over a period of 15 or more years. The two hypotheses receiving the most serious consideration are that the PN antigen is (1) a viral antigen or (2) an antigen that appears in response to the interruption of hepatocyte differentiation seen in the nodule populations. Regardless of its fundamental nature, the observations on the PN antigen offer the strongest evidence of a unifying link between the hyperplastic hepatocyte populations presumed to be premalignant and the malignant neoplastic hepatocytes in liver cancer. More importantly, this antigen offers a new marker which could prove useful as a quantitative index of early changes related to cancer development.

REFERENCES

Becker, F.F. "Alteration of Hepatocytes by Subcarcinogenic Exposure to N-2-Fluorenylacetamide," Cancer Res. 35:1734-1736, 1975. (CA-15859)

Cohen, N.D. and Hilf, R. "Influence of Insulin on Estrogen-induced Responses in the R3230AC Mammary Carcinoma," Cancer Res. 35:560-567, 1975. (CA-16660)

Costlow, M.E., Buschow, R.A., Richert, N.J. and McGuire, W.L. "Prolactin and Estrogen Binding in Transplantable Hormone-dependent and Autonomous Rat Mammary Carcinoma," Cancer Res. 35:970-974, 1975. (CA-11378)

Cox, R. and Irving, C.C. "Damage and Repair of DNA in Various Tissues of the Rat Induced by 4-Nitroquinoline 1-Oxide," Cancer Res. 35:1858-1860, 1975. (CA-15189)

Herzog, J., Serroni, A., Briesmeister, B.A. and Farber, J.L. "N-Hydroxy-2-acetylaminofluorene Inhibition of Rat Liver RNA Polymerases," Cancer Res. 35:2138-2144, 1975. (CA-12073)

Horwitz, K.B., McGuire, W.L., Pearson, O.H. and Segaloff, A. "Predicting Response to Endocrine Therapy in Human Breast Cancer: A Hypothesis," Science 189:726-727, 1975. (CA-11378)

King, C.M. and Olive, C.W. "Comparative Effects of Strain, Species, and Sex on the Acyltransferase- and Sulfotransferase-catalyzed Activations of N-Hydroxy-N-2-fluorenylacetamide," Cancer Res. 35:906-912, 1975. (CA-13179)

King, C.M., Olive, C.W. and Cardona, R.A. "Activation of Carcinogenic Arylhydroxamic Acids by Human Tissues," J. Natl. Cancer Inst. 55:285-287, 1975. (CA-13179, CA-15640)

Kitagawa, T., Michalopoulos, G. and Pitot, H.C. "Unscheduled DNA Synthesis in Cells from N-2-Fluorenylacetamide-induced Hyperplastic Nodules of Rat Liver Maintained in a Primary Culture System," Cancer Res. 35:3682-3692, 1975. (CA-07175)

Kitagawa, T. and Pitot, H.C. "The Regulation of Serine Dehydratase and Glucose-6-phosphatase in Hyperplastic Nodules of Rat Liver during Diethylnitrosamine and N-2-Fluorenylacetamide Feeding," Cancer Res. 35:1075-1084, 1975. (CA-07175)

LaMont, J.T. and Isselbacher, K.J. "Alterations in Glycosyltransferase Activity in Human Colon Cancer," J. Natl. Cancer Inst. 54:53-56, 1975. (CA-14294)

Lin, J.-K., Miller, J.A. and Miller, E.C. "Structures of Hepatic Nucleic Acid-bound Dyes in Rats Given the Carcinogen N-Methyl-4-aminoazobenzene," Cancer Res. 35:844-850, 1975a. (CA-07175, CA-15785)

Lin, J.-K., Schmall, B., Sharpe, I.D., Miura, I., Miller, J.A. and Miller, E.C. "N-Substitution of Carbon 8 in Guanosine and Deoxyguanosine by the Carcinogen N-Benzoyloxy-N-methyl-4-aminoazobenzene in Vitro," Cancer Res. 35:832-843, 1975. (CA-07175, CA-15785, CA-02332)

Maher, V.M., Birch, N., Mittlestat, M., Otto, J., Queliette, L., Schnur, T. and McCormick, J.J. "Effect of Excision and Post-Replication DNA Repair on the Cytotoxicity and Mutagenicity of UV in Human Skin Fibroblasts," Proc. Am. Assoc. Cancer Res. 158, 1975. (CA-13058, CA-14680)

Maher, V.M. and McCormick, J.J. "Effect of DNA Repair on the Cytotoxicity and Mutagenicity of UV Irradiation and of Chemical Carcinogens in Normal and Xeroderma Pigmentosum Cells," in The Biology of Radiation Carcinogenesis, J. Yuhas, ed., Raven Press, New York, 1975a. (CA-13058)

Maher, V.M. and McCormick, J.J. "Cytotoxic and Mutagenic Effects of Carcinogenic Aromatic Amides and Polycyclic Hydrocarbons and Ultraviolet Irradiation in Normally Repairing and Repair-Deficient (Xeroderma Pigmentosum) Diploid Human Skin Fibroblasts," in Molecular Mechanisms for the Repair of DNA, P. Hanawalt and R. Setlow, eds., pp. 785-787, Plenum Press, New York, 1975b. (CA-13058)

Maher, V.M. and Wessel, J.E. "Mutations to Azaguanine Resistance Induced in Cultured Diploid Human Fibroblasts by the Carcinogen, N-Acetoxy-2-Acetylaminofluorene," Mutation Res. 28:277-284, 1975. (CA-14680, CA-13058)

McGuire, W.L. "Current Status of Estrogen Receptors in Human Breast Cancer," Cancer 36:638-644, 1975. (CA-11378)

O'Brien, T.G., Simsiman, R.C. and Boutwell, R.K. "Induction of the Polyamine-biosynthetic Enzymes in Mouse Epidermis by Tumor-promoting Agents," Cancer Res. 35:1662-1670, 1975a. (CA-07175, T1-CA-05002)

O'Brien, T.G., Simsiman, R.C. and Boutwell, R.K. "Induction of the Polyamine-biosynthetic Enzymes in Mouse Epidermis and Their Specificity for Tumor Promotion," Cancer Res. 35:2426-2433, 1975b. (CA-07175, T1-CA-05002)

Okita, K., Kligman, L.H. and Farber, E. "A New Common Marker for Premalignant and Malignant Hepatocytes Induced in the Rat by Chemical Carcinogens," J. Natl. Cancer Inst. 54:199-202, 1975. (CA-12218, CA-12227, T1-CA-5280)

Schumm, D.E. and Webb, T.E. "Modification of Nuclear Restriction In Vitro by Plasma From Tumor-Bearing Animals," J. Natl. Cancer Inst. 54:123-128, 1975. (CA-12411)

Swenberg, J.A., Koestner, A., Wechsler, W., Brunden, M.N. and Abe, H. "Differential Oncogenic Effects of Methylnitrosourea," J. Natl. Cancer Inst. 54:89-96, 1975. (CA-11224)

Trosko, J.E., Yager, J.D., Jr., Bowden, G.T. and Butcher, F.R. "The Effects of Several Croton Oil Constituents on Two Types of DNA Repair and Cyclic Nucleotide Levels in Mammalian Cells in Vitro," Chem.-Biol. Interactions 11:191-205, 1975. (CA-13048, CA-07175)

Wislocki, P.G., Miller, J.A. and Miller, E.C. "The Carcinogenic and Electrophilic Activities of N-Benzoyloxy Derivatives of N-Methyl-4-aminoazobenzene and Related Dyes," Cancer Res. 35:880-885, 1975. (CA-07175, CA-15785)

Yager, J.D., Jr. and Potter, V.R. "A Comparison of the Effects of 3'-Methyl-4-dimethylaminoazobenzene, 2-Methyl-4-dimethylaminoazobenzene, and 2-Acetylaminofluorene on Rat Liver DNA Stability and New Synthesis," Cancer Res. 35:1225-1234, 1975. (CA-07175)

Yost, Y., Gutmann, H.R. and Rydell, R.E. "The Carcinogenicity of Fluorenylhydroxamic Acids and N-Acetoxy-N-fluorenylacetamides for the Rat as Related to the Reactivity of the Esters toward Nucleophiles," Cancer Res. 35:447-459, 1975. (CA-02571)

PROJECTIONS

The emphasis of this program is on Environmental Carcinogenesis, with particular focus on the need to expand the scope and the amount of research in the following areas: (1) mechanisms of action of chemical carcinogens, (2) modification of host response to chemical carcinogens and sunlight (UV), and (3) assessment of the role of promoters in environmental carcinogenesis.

Studies on the mechanisms of action of chemical carcinogens are fundamental to the achievement of some of the prime cancer research objectives, such as: (a) the development of in vitro carcinogenicity screening methods and systems, (b) the development of approaches to the preclinical detection of cancer, and (c) the possible use of dietary, drug, and/or hormonal means of protecting individuals against the effects of exposure to environmental carcinogens. This same research area addresses itself to the question of the extent to which chemical carcinogenesis is a genetic or epigenetic phenomenon.

In the endeavor to protect people against cancer due to environmental agents, it is clearly of great importance to identify as many chemical carcinogens as possible and to seek to remove them from the environment. It is to be expected, however, that many such agents might remain unidentified for a long time and that it would be very difficult to rid the environment of many of those which are known. Hence it is of interest to explore ways in which the neoplastic effects of chemical carcinogens in the host might be inhibited, possibly by dietary, pharmacologic, or hormonal means, or by combinations of these approaches; e.g., studies on the experimental inhibition of hydrocarbon-induced carcinogenesis by the use of antioxidants. In this context, the present focus is on the protection

of human populations against the effects of chronic exposure to low doses of environmental carcinogens.

At the present time, it is not clear whether the concept of chemical carcinogenesis as a two-stage process (initiation, promotion) has general application to organs other than skin and to humans. If so, then the role of promoters in environmental carcinogenesis could be expected to be far-reaching, since initiation with subcarcinogenic doses of chemical carcinogens is expected to occur in the population-at-large.

CANCER EPIDEMIOLOGY PROGRAM

Genrose D. Copley, M.D., Program Director

HISTORY AND CONTENT OF PROGRAM

The description and measurement of cancer, its classification by type and extent, and the dual searches for cause and measurement of treatment effect in human populations are not new activities. They have always constituted a small but important percentage of the total cancer effort. In 1967, this program reported 24 research grants, totaling $1,464,000 and constituting 1.9 percent of the research budget. Funding decreased to less than $500,000 in 1969, and since has grown gradually in dollar amount. In 1975, approximately $7.945 million was invested in 56 research grants, and $8.132 million in 54 grants in 1976. This is less than one percent of funds available for unsolicited research grants, approximately half the proportion in 1967. The failure to keep pace with the growth of other areas of research in cancer is a reflection of the serious problems which confront epidemiology and related disciplines, not a lack of research potential. Indeed the potential for research has grown with the development of data resources, the establishment of cancer control programs and cancer centers, and the expansion of basic and clinical research. The most serious unresolved problem is a shortage of personnel at the senior investigator level.

One of the problems and, at the same time, strong points is the dynamic quality of the program. Of the 51 regular grants, 22 are in their first year and 9 in their second. Only seven are more than five years old, and these include support for the International Union Against Cancer (UICC), the journal Cancer Research, the American Joint Committee on End Results and Cancer Staging, and three biometric resources. One new program project replaces one phased out.

Relative emphasis on epidemiology, biometry, and population genetics has strengthened. With the development of psychosocial interventive programs, pain control, and rehabilitation in the Division of Cancer Control and Rehabilitation, service-oriented investigations have found another source of support. Research applications related to psychosocial factors in cancer etiology have not been favorably reviewed because their design has not been sufficiently rigorous.

Epidemiologic studies continue to dominate the program. Among the active grants, there is heavy concentration on studies of cancer in females--cervix, breast, ovaries, and endometrium. This interest has occurred spontaneously.

Thirteen grants deal with various aspects of these cancer sites and consider diet, hormones, chemicals,and viruses as potential etiologic agents. Many of these grants are complementary. In addition, five of the seven program project grants support investigations in these areas.

RESEARCH ACCOMPLISHMENTS

Differences in cancer experience among people with varied genetic backgrounds are frequently studied to elucidate the effects of environmental or of genetic factors. In a study of cancer incidence in Los Angeles County (Menck, 1975), Mexican-Americans of both sexes were found to have lower incidence than other Caucasians for cancers of all sites combined. Buccal cavity, colon, rectum, larynx, lungs, breast, bladder, prostate, and testis were specific sites of lower incidence whereas stomach, gall bladder, liver, and uterine cervix were higher in Mexican-Americans than in other whites. Immigrant rates were most divergent, with indigenous Mexican-Americans intermediate. The cancer patterns in the Mexican-Americans were similar to observations in American Indians. This is thought to be consistent with environmental variations in cause.

In other studies, however, it appears that variations in genetic background may be strongly associated with increased cancer risk. Persons with certain autosomal recessive disease syndromes such as Fanconi's anemia and ataxia telangiectasia seem to have an excess risk of cancers and leukemia. In a study of eight families of Fanconi anemia patients, it was found that such heterozygote relatives may have a risk of dying from malignant neoplasia three times that of the general population (Swift, 1975). These deaths occurred mostly in 50-69 year olds, and leukemia, pharyngeal, gastrointestinal, and bladder malignancies occurred more frequently than expected. It is estimated that heterozygotes for Fanconi's anemia occur with a frequency of one in 300 persons, so that Fanconi anemia heterozygotes may constitute one percent of all cancer deaths, with the proportions varying by specific neoplasm.

Heterozygotes for ataxia telangiectasia are thought to constitute up to one percent of the general population. In a study of 27 families of patients with this condition, deaths from malignant disease were compared with the experience of the general population (Swift et al., 1975). The overall risk of cancer death was significantly increased ($p < 0.02$), but the risk of dying of cancer prior to age 45 was five times that of the general population. Ovarian, gastric, biliary system cancers, leukemia, and lymphomas occurred more frequently. Other neoplasias which may be associated are pancreatic, basal cell, colonic, breast, and cervical cancer. The risk of malignant disease increased with increasing likelihood of heterozygosity.

Genetic factors are frequently postulated to influence breast cancer incidence. For example, there is less change in incidence with modified environment than is exhibited by some other neoplasia such as colon cancer. Genetic factors may damp the effect of environmental change. While much is known about the physiology and secretory activity of lactating breasts, until recently there had been little objective research concerned with the normal human nonlactating breast. Biologic and physiologic factors associated with breast fluids obtained by a nipple aspiration from 606 normal nonlactating women were reported this year (Petrakis, 1975). Data on race, age, menstrual status, parity, contraceptive pill use, hormone use, and cerumen type indicated that the availability of secretions was related to race, age, and menopause and to genetic factors associated with apocrine gland function.

Fluid aspirates were obtained from approximately 70 percent of Caucasians and about 24 percent of Chinese. The percent of successful aspirations decreased in members of all races over 50 years of age, but less so among Caucasians. Chinese and Japanese women with dry cerumen yielded a lower percentage of successful aspirations than those with wet type cerumen, suggesting that genetic factors may be associated with breast fluid secretion in nonlactating women.

Cellular findings in aspirate specimens from 796 women yielded 50 women with abnormal cells and/or microcalcifications (King, 1975). Abnormal cells were found in 50 percent of the satisfactory specimens from women who had breast cancer or had previously had a mastectomy for breast cancer. Continued observation of such women for evidence of regression, persistence,or progression of cytologic abnormalities is required to determine the significance of the abnormal cells. Microcalcifications can be detected both in nipple aspirates and by mammography, although results of the two techniques do not always agree in each individual case. The absence of mammographic confirmation of cytologic findings of microcalcifications may be an indication for reevaluating existing mammograms and for repeat clinical and mammographic examination at more frequent intervals for early localization of small lesions.

Another subject of continuing interest has been the relationship between reserpine and breast cancer. Two studies (O'Fallon, 1975; Mack, 1975) failed to confirm the findings of three earlier studies suggesting increased risk of breast cancer in women taking Rauwolfia derivatives. It was concluded that they are not likely to cause breast cancer.

Exogenous hormones administered therapeutically or for fertility control have also been suspected of a role in breast cancer etiology.

The relationship between breast cancer or benign breast disease and oral contraceptive use was sought in a study of 1,770 women under 50 years of age, including 452 with breast cancer and 446 with benign breast disease. Although the relative risk of breast cancer among women who had ever used birth control pills was 1.1, the risk increased significantly to 1.9 or more among women who had used the pills for 2-4 years. A history of benign breast disease increased the cancer risk among long-term users by as much as eleven-fold. Breast cancer risk did not vary by age, age at which use of pills began, or interval since first or last use. These results could be interpreted to indicate that oral contraceptives did not induce breast cancer but may have accelerated the growth rate of preexisting cancer (Fasal, 1975).

A reduced rate of benign breast disease with increasing time on oral contraceptives was also found, suggesting a protective effect for oral contraceptives against benign breast disease. This finding, and the lack of cancer causing role for oral contraceptives was confirmed by a prospective study, also reported in 1975 (Ory). In the greater Boston area, 97,254 married women between 25 and 49 years of age were followed prospectively for 30 months for breast disease, and there were 1,072 hospitalizations (Ory, 1975). Women who had taken oral contraceptive pills for up to 12 months and non-users had similar hospitalization rates. Women who had used oral contraceptives for 13-24 months or more than two years were hospitalized for breast disease 70 percent and 35 percent, respectively, as frequently as non-users. The use of oral

contraceptives appears to lower the risk of both fibrocystic disease and fibroadenoma. While the rate of breast cancer was lower among users than non-users, the difference was neither statistically significant nor related to duration of use.

The relation of oral contraceptives to other neoplasia is also interesting. A study of 42 women with histologically proven liver adenomas since 1955 in Los Angeles (Edmonson, 1975) revealed a significant difference ($p < 0.001$) from neighborhood controls in mean months of oral contraceptive usage. This relationship was enhanced with respect to mesantrol-containing pill use ($p < 0.0001$). Hemorrhage into the tumor was often associated with the onset of menstruation in women using oral conceptives. The risk increased sharply beyond five years of pill use.

A follow-up of 110 girls born of women who received stilbestrol during pregnancy reveals a high incidence of benign alterations of the genito-urinary tract, including transverse ridges (22%), abnormal vaginal mucosa (35%), and biopsy proven adenosis (35%) (Herbst, 1975). Among 82 unexposed girls, there were no ridges and only one case of vaginal mucosal abnormality, including adenosis. Abnormal cervical epithelium occurred in almost all exposed subjects but in only half the controls. The incidence of vaginal abnormalities was highest when diethylstilbestrol was begun in early pregnancy. No cases of cancer were observed.

Atmospheric environmental agents, specifically polynuclear aromatic hydrocarbons, have been reported to be associated with lung cancer (Henderson, 1975). Between 1968 and 1972, an excess of lung cancers was consistently observed in men in south central Los Angeles. This excess persisted across several occupational categories and social classes, but there was no differential excess risk of cancers of other sites associated with cigarette smoking. Air samples from the area contained excesses of certain polynuclear aromatic hydrocarbons.

Investigations into the time-dose relationships between radiation exposure and cancer incidence continue to reveal new risks. A second follow-up study of children treated by x-ray epilation for ringworm of the scalp during the years 1940-1955 has been undertaken (Shore, 1975). The comparison group were age-, sex-, and race-matched controls treated by other means. There were excess incidences of tumors of the head and neck in the irradiated group, including skin, brain, thyroid, and parathyroid. All observed cases of leukemia occurred within the first twenty years. No differences in death rate between the irradiated and control groups were observed.

Association between animal and human tumor experience has been sought in numerous instances, especially with regard to leukemias. Animal tumor experience may also provide leads to human cancer behavior, but relatively little is known about naturally occurring tumors. In the San Francisco area, there are both human and animal tumor registries. It is much easier to establish human cancer rates than animal rates because the animal population in a given area is usually unknown. In 1970, a sample household survey was carried out to estimate the animal population in the area (Schneider, 1975). It was found that there was one dog for every 7.3 persons and one cat for every 10.8 persons. Almost half

the households included at least one dog or one cat. From proportional distribution by age, it appeared the growth in canine population may have peaked in 1968. Less than half the bitches (47.8%) and more than half the queens (64.6%) were neutered. The highest reproducing ages for both bitches and queens were between one and three years of age, with an average of 0.4 litters per intact bitch and 1.6 litters per intact queen. No information is provided about the age or number of humans sharing quarters with the animals.

REFERENCES

Edmonson, H.A., Henderson, B. and Benton, B. "Liver-Cell Adenomas Associated with Use of Oral Contraceptives," New Eng. J. Med. 470-472, February 26, 1976. (CA-17054)

Fasel, E. and Paffenbarger, R., Jr. "Oral Contraceptives as Related to Cancer and Benign Lesions of the Breast," J. Natl. Cancer Inst. 55:767-773, 1975. (CA-17838)

Henderson, B.E., Gordon, R.J., Menck, H., Sookoo, J., Martin, S.P. and Pike, M. "Lung Cancer and Air Pollution in South Central Los Angeles County," Am. J. Epid. 106:477-488, 1975. (CA-17054)

Herbst, A.L., Poskanzer, D.C., Robboy, S.J., Friedlander, L. and Scully, R.E. "Prenatal Exposure to Stilbestrol: A Prospective Comparison of Exposed Female Offspring with Unexposed Controls," New Eng. J. Med. 292:334-339, 1975. (CA-13139)

King, E.B., Barrett, D. and Petrakis, N. "Cellular Composition of the Nipple Aspirate Specimen of Breast Fluid," Am. J. Clin. Path. 64:739-748, 1975. (CA-13556)

Mack, T.H., Henderson, B., Gerkins, V.R., Arthur, M., Baptista, J. and Pike, M. "Reserpine and Breast Cancer in a Retirement Community," New Eng. J. Med. 292:1336-1371, 1975. (CA-17054)

Menck, H.R., Henderson, B.E., Pike, M.C., Mack, T., Martin, S.P. and Sookoo, J. "Cancer Incidence in the Mexican American," J. Natl. Cancer Inst. 53:531-536, 1975. (CA-17054)

O'Fallon, W.M., Labothe, D.R. and Kurland, L.T. "Rauwolfia Derivatives and Breast Cancer," The Lancet II:292-296, 1975. (F22-CA-02204)

Ory, H., Cole, P., MacMahon, B. and Hoover, R. "Oral Contraceptives and Reduced Risk of Benign Breast Diseases," New Eng. J. Med. 294:419-422, 1976. (CA-06373)

Petrakis, N.L., Mason, L., Lee, R., Seegimoto, B., Pawson, S. and Catchpool, F. "Association of Race, Age, Menopausal Status and Cerumen Type With Breast Fluid Secretion In Nonlactating Women as Determined by Nipple Aspiration," J. Natl. Cancer Inst. 54:829-834, 1975. (CA-13556)

Schneider, R. and Vaida, M.L. "Survey of Canine and Feline Population: Alameda and Contra Costa Counties, California, 1970," J. Am. Vet. Med. Assn. 166:481-486, 1975. (CA-14916)

Shore, R.E., Albert, R.E. and Pasternak, B.S. "Follow-Up Study of Patients Treated by X-Ray Epilation for Tinea Capitis," Arch. Env. Health 31:17-24, 1976. (CA-17188)

Swift, M. "Fanconi Anemia: Cellular Abnormalities and Clinical Predisposition to Malignant Disease," in Congenital Disorders of Erythropoiesis, Ciba Foundation Symposium 37 (new series), Elsevier-Excerpta Medica, North-Hollywood, January 1976. (CA-14235)

Swift, M., Sholman, L., Perry, M. and Chase, C. "Malignant Neoplasms in the Families of Patients With Ataxia Telangiectasia," Cancer Res. 36:209-215, January 1976. (CA-14235)

PROJECTIONS

With the strong emphasis on environmental and genetic factors in the current publications, it appears that work in these two areas will be expanded and elaborated. The interactions between extrinsic and intrinsic factors are likely to be of increasing interest.

Studies likely to be reported include: findings associated with exposure to low-dosage radiation, both with respect to direct exposure and delayed effects from germinal tissue exposure; cancer deaths associated with arsenic exposure; other diseases associated with leukemia; secretion of environmentally derived substances into nonlactating breast fluids; further investigation into the association between viral infection and dysplasia, the progression of dysplasia and the relationship of both to carcinoma in situ of the uterine cervix; and comparison of veterinarians' and physicians' malignancy deaths.

OTHER ACTIVITIES

A population-based registry in Los Angeles County in support of USC-UCLA Cancer Centers is compatible with the SEER data, but uniquely suited to the needs of the cancer center. Information about patients with various types of neoplasia is available in time to permit selection of matched controls, collection of specimens for laboratory or research purposes, and interview. An active biometry and epidemiology research staff on site analyze the data and assist in the evaluation of center programs. The registry is integrated with the medical school, cancer center and UCLA School of Public Health. In addition, two animal tumor registries are supported--one in the San Francisco Bay area, permitting correlation between animal and human cancers on a geographic basis. Two data-linkage studies are supported. One provides a research resource--the linkage of Mormon genealogic records with the Utah Tumor registry; the other links AEC employment records with Social Security death and disability records.

The grants program contributes in another general way to the data system. Two grants support efforts to improve the specificity and accuracy of medical records in the United States and internationally. These support the American Joint Committee on Cancer Staging and End Results (AJC) reporting in the United States and the TNM classification activities of the International Union Against Cancer (UICC). There is a liaison between the two organizations and both have recently increased their tempo. The Joint Committee also interacts with the Surveillance, Epidemiology, and End Results Reporting (SEER) Program and the Cancer Center Data System. In March 1976, the first joint meeting of task force members was held and a report to the professions of the American Joint Committee was made. A draft manual reporting the current recommendations for cancer recording was available for review. It is hoped that a manual suitable for distribution and medical records use will be available by June. This

manual will probably reflect increasing acceptance of modifications of the TNM system for records maintenance and data management. It also appears that cancer journals will adopt the AJC recommendations as a standard for published papers, and will encourage other journals publishing large numbers of cancer related reports to follow suit.

Two activities supported by NCI encourage the development and centralization of comparable data by cancer centers. These are the Cancer Center Data System, intended to provide NCI with information about patients treated by Cancer Centers, and the Cancer Center Epi-Stat Units developed under the aegis of the Division of Cancer Research Resources and Centers (DCRRC), Cancer Centers Program. At least marginal statistical resources have been developed in each comprehensive cancer center. The first meeting of these personnel indicated interest in collaborating on solving several problems. These include the development of methodology for cancer center self-evaluation, collaborative etiologic studies, compatible data collection units concerned with epidemiologic data, and training. In some instances, epidemiologic staff are also available and active--notably at USC, Seattle, and Rochester, Minnesota.

Coordination with intramural cancer epidemiology and biometry programs is accomplished largely through informal staff meetings and discussions and through mutual attendance of review committee meetings. In a number of research subject indexes recently developed, cancer epidemiologic and biometric projects tend to be integrated by subject with other research. There is a joint project to develop a listing (by principal investigator, subject and funding agency) of ongoing or recently completed research. This is to be used in planning, program development, and coordination.

VIRAL ONCOLOGY PROGRAM

Philip G. Stansly, Ph.D., Program Director

HISTORY AND CONTENT OF PROGRAM

The National Cancer Institute has supported research in cancer virology by means of grants since its inception. For example, investigations concerning the mouse mammary tumor agent and the rabbit papilloma virus were supported by grants as early as 1938. The discovery of mouse leukemia as well as polyoma viruses in 1951 enormously stimulated the field and resulted in a large increase in grant-supported research in virology. In 1969, Viral Oncology became the major component of a Cancer Biology program. In 1974, Viral Oncology was separated out as an independent program.

The original goal of grant support for virology was to rapidly expand our knowledge of the occurrence, nature and mechanisms of virus-induced cancer. It was hoped that such knowledge would be relevant to human cancer and would contribute to improved methods of diagnosis, prevention, and treatment of neoplasia.

From the beginning, the virology grants program contributed important information not only to cancer virology, as expected, but to other cancer research areas and to biomedicine generally. These contributions include the demonstration of the viral etiology of mouse mammary cancer, the discovery of in vitro transformation by tumor viruses, the discovery of the integration of viral and cellular genes and of reverse transcriptase. Contributions to general cancer research and biomedicine include the development and application of cell hybridization to mammalian genetics and experimental oncology, the development and application of molecular hybridization to viral oncology and genetic chemistry, and the use of restriction enzymes to analyze genes for structure and function.

Many of the above examples also illustrate a fact that is not always evident or appreciated, i.e., the contribution of cancer research to basic biomedical research, particularly cell biology. Examples which may be cited, in addition to those above, are the enormous impact of reverse transcriptase on molecular biology and genetics, and the induction of differentiation in virus-induced hemoblasts by dimethylsulfoxide, which provides the opportunity to study hemopoiesis in vitro.

The Viral Oncology program of grant-supported research currently emphasizes the biology of cell-virus interaction, the nature and mechanisms of virus-induced neoplastic transformation, and the alterations in the cell that characterize it as neoplastic. This emphasis broadly serves to distinguish the grant-supported from the complementary contract-supported program, which focuses on the role of viruses in human cancer.

The identification of viral genes and their products, which are responsible for neoplastic transformation and for the maintenance of the transformed state, are primary targets of the program. It is desired to learn how these genes or

their products interact with cellular components, especially cellular genes, and which cellular genes are so involved. The consequences of these interactions on the appearance of new cellular functions are further objectives of this program.

Because of their intimate interaction with cellular components and because they provide a handle or marker which permits one to follow these reactions, tumor viruses are considered basic tools to study how and why certain genes of a cell are expressed and others repressed, and thus how various functions of the cell are controlled during normal growth and differentiation and in malignant transformation. Such studies are vital to our understanding of neoplastic transformation, which results in new cellular functions. Insight of this sort may also suggest strategies to counteract these abnormal functions and reprogram the malignant cell so that it is again responsive to normal growth controls.

The program currently consists of 272 traditional grants and 23 program projects, distributed as follows:

272	Traditional Research Projects	
116	RNA Tumor Virus Projects	$ 8.338 million
108	DNA Tumor Virus Projects	7.487 million
48	Basic and Supportive Studies	3.304 million
23	Program Projects and Centers	13.096 million
295	Total	$32.225 million

About 70 percent of the research supported by Viral Oncology Program relates to Cause and Prevention (Thrust 1 of the National Cancer Plan). The balance falls under the category of Cancer Biology (Thrust 4).

RESEARCH ACCOMPLISHMENTS

Tumorigenicity of SV40-transformed cells in nude mice is specifically correlated with anchorage-independent growth in vitro. Other cellular parameters of virus-induced transformation, such as lack of sensitivity to high cell density or the capacity for the cells to grow in low serum concentration are dissociable from tumorigenicity (Shin et al., 1975).

In vitro transformation of lymphoid cells was achieved with a leukemia virus (Rosenberg et al., 1975). This is the first instance of such transformation by a leukemia virus (Abelson strain) and indicates that lymphoid cells are indeed the target cells for oncogenic transformation by these viruses. The transformed cells were oncogenic in adult BALB/c mice.

Bishop et al. (1975) have demonstrated the existence of and, in effect, isolated the transforming gene of the avian sarcoma virus. They have prepared,

in a series of adroit procedures, a DNA sequence complementary to the deleted nucleotide sequence of an avian sarcoma virus defective for transformation. This DNA ("cDNA sarc") is therefore thought to be the transforming gene, inasmuch as the genomes of all transforming avian viruses contain sequences complementary to "cDNA sarc," whereas such sequences are missing from the leukosis viruses (which are non-transforming) as well as from transformation-defective avian sarcoma mutants. They have further found that these DNA sequences occur in all avian species tested, even in the Japanese quail, for which no C type virus has as yet been demonstrated in adults, although some spontaneous expression of a C type virus occurs in embryos. It is of extraordinary interest that transcription of this gene occurs in a methylcholanthrene-induced tumor of the Japanese quail. A tentative conclusion is that all avian cells have the gene for neoplastic transformation which can be activated under certain circumstances, e.g., by methylcholanthrene.

An avian sarcoma virus passed through duck cells will acquire duck genetic information, which becomes covalently linked to its RNA (Shoyab et al., 1975). Thus, genetic alteration of RNA tumor viruses by acquisition of host cell genetic information (possibly host endogenous virus sequences) may be involved in tumor virus formation and in information transfer from cell to cell. It has previously been found by others (e.g., Winocour et al., 1975) that analogous events occur with SV40 DNA.

By genetic and biochemical analysis, a portion of the circular, double-stranded genome of SV40 (the early "A" region) was shown to be responsible for three functions of the virus: replication, initiation, and maintenance of transformation. More recent results have focused on at least one likely product of this gene, the T antigen. This is a protein that appears early after infection of cells with SV40 (related T antigens are found with certain other virus infections) in either transforming or replicating systems and may be the long sought protein responsible for transformation. Jessel et al. (1975) have detected a property of the T antigen which should advance our understanding of its function; this is its quantitative binding to certain specific segments of the SV40 genome. This finding will allow the preparation of purified T antigen and expedite the investigation of this protein.

It seems likely now that infectious DNA transcripts of the RNA tumor viruses will eventually be synthesized in vitro. Previously, only short-length DNA sequences could be identified in reverse transcript reactions, and infectious DNA could be isolated only from infected cells. Junghans et al. (1975) have synthesized full-length transcripts of Rous sarcoma RNA simply by improving the in vitro conditions - especially inhibiting the action of nucleases. This is a first step towards synthesizing infectious transcripts for genetic, biochemical, and functional analyses similar to those being so successfully carried out with the DNA viruses.

The intimate relation of the RNA tumor virus to the cell it infects is indicated by the finding that the "primer" which starts the synthesis of viral DNA is a host transfer RNA, thus indicating at least one aspect of host control over RNA tumor virus infection (Harada et al., 1975).

Etienne Lasfargues et al. (1976) have found that normal cat cells can be infected with the virus of mouse mammary tumor. This is the first time that a

cell has been exogenously infected with this agent and opens the way to a controlled molecular study of this virus, hitherto hampered for lack of a suitable experimental system.

Robert Nowinski et al. (1975) have discovered that, contrary to widespread belief, mice naturally have antibodies to their endogenous leukemia virus. If the disease can be prevented in mice by augmenting this natural response, there will be added encouragement to investigate this approach for the prevention of the human disease.

The human wart virus, a papova virus, is the only confirmed human tumor virus. Although skin warts and related papillomas are "benign" tumors (though they can still be life threatening in some circumstances), the possibility that they could progress to malignancy must be considered, especially as other papova viruses can induce malignant transformation. The recent report of the propagation of the wart virus (Eisinger et al., 1975) will allow the preparation of the necessary reagents to study in depth the virus and its relation to human cancer.

The widespread occurrence of a glycoprotein (gp 69/71) on the surface of certain normal murine and avian cells, and even in the serum of these species, has been observed (Tung et al., 1975). This moiety appears to be identical with the envelope glycoprotein of the leukemia-sarcoma viruses and therefore probably represents an expression of the integrated viral DNA. It seems possible that it may provide a useful marker for the presence of the tumor virus genome and as such has potential for etiological, epidemiological, and diagnostic studies in humans.

REFERENCES

Bishop, J.M., Deng, C.T., Moscovici, C., Stavnezer, E., Stehelin, H.E., Varmus, H. and Vogt, P. "Avian RNA Tumor Viruses: Viral Gene Expression and Cellular Transformation," Abstracts, 3rd International Congress for Virology, 32, Madrid, September 10-17, 1975. (CA-12705, CA-10697)

Eisinger, M., Kucarova, O., Sarkar, N.H. and Good, R.A. "Propagation of Human Wart Virus in Tissue Culture," Nature 256:432-434, 1975. (CA-17129)

Harada, F., Sawyer, R.C. and Dahlberg, J.E. "A Primer Ribonucleic Acid for Initiation of In Vitro Rous Sarcoma Virus Deoxyribonucleic Acid Synthesis," J. Biol. Chem. 250:3487-3497, 1975. (CA-15166)

Jessel, D., Hudson, J., Landau, T., Tenen, D.G. and Livingston, D.M. "Interaction of Partially Purified Simian Virus 40 T Antigen with Circular Viral DNA Molecules," Proc. Nat. Acad. Sci. 72:1960-1964, 1975. (CA-15751)

Junghans, R.P., Duesberg, P.H. and Knight, C.A. "In Vitro Synthesis of Full-Length DNA Transcripts of Rous Sarcoma Virus RNA by Viral DNA Polymerase," Proc. Nat. Acad. Sci. 72:4895-4899, 1975. (CA-11426)

Lasfargues, E.Y., Lasfargues, J.C., Dion, A.S., Greene, A.E. and Moore, D.H. "Experimental Infection of a Cat Kidney Cell Line with the Mouse Mammary Tumor Virus," Cancer Res. 36:67-72, 1976. (CA-08515)

Nowinski, R.C., Kaehler, S.L. and Burgess, R.R. "Immune Response in the Mouse to Endogenous Leukemia Viruses," Cold Spring Harbor Symp. Quant. Biol. 39:1123-1128, 1975. (CA-07175)

Rosenberg, N., Baltimore, D. and Scher, C.D. "In Vitro Transformation of Lymphoid Cells by Abelson Murine Leukemia Virus," Proc. Nat. Acad. Sci. 72:1932-1936, 1975. (CA-12174)

Shin, S.I., Freedman, V.H., Risser, R. and Pollack, R. "Tumorigenicity of Virus-Transformed Cells in Nude Mice is Correlated Specifically with Anchorage Independent Growth In Vitro," Proc. Nat. Acad. Sci. 72:4435-4439, 1975. (CA-13106)

Shoyab, M., Markham, P.D. and Baluda, M.A. "Host Induced Alteration of Avian Sarcoma Virus B-77 Genome," Proc. Nat. Acad. Sci. 72:1031-1035, 1975. (CA-10197)

Tung, J.S., Vitetta, E.S., Fleissner, E. and Boyse, E.A. "Biochemical Evidence Linking G IX Thymocyte Surface Antigen to the gp 69/71 Envelope Glycoprotein of Murine Leukemia Virus," J. Exp. Med. 141:198-205, 1975. (CA-15297)

Winocour, E., Frenkel, N., Lavi, S., Osenholts, M. and Rozenblatt, S. "Host Substitution in SV40 and Polyoma DNA," Cold Spring Harbor Symp. Quant. Biol. 39:101-108, 1975. (CA-14530)

PROJECTIONS

The following types of investigation are the most pressing and significant for continued and future investigations in the Viral Oncology area:

1. Analysis of the genomes of tumor-inducing viruses to determine the position, structure, and function of their constituent nucleic acid sequences.
2. Identification and isolation of the sequence(s) responsible for transformation. In the case of the RNA viruses, this endeavor also includes the mechanism of formation of the DNA transcript of the virus, for this is its true genetic substance.
3. The mechanism of integration and excision of viral genes from the cell genome.
4. The events which follow integration, i.e., there may be either no discernible expression, minimal expression (e.g., some messenger), or transformation with or without virus replication. These occurrences are followed by analyzing transcription and translation events (in cell or cell-free systems) together with chemical and functional analysis of the various products - i.e., messengers and proteins. It is generally thought that some protein product of a messenger from the integrated viral genome is responsible for transformation. Such products are being widely sought but have not yet been identified with certainty.
5. Analysis of the structure and function of components of tumor viruses other than their genomes. Such studies are important in providing markers for ecological and epidemiological studies of tumor viruses (and their expressions), in determining relationships among them, and in providing potential reagents for diagnosis and for active and passive immunization.
6. The role of tumor virus information in apparently normal cells: Are these really normal constituents? If so, are so-called tumor viruses essentially carriers of oncogenic information which is really cellular in origin? How is this information activated? What is its significance for oncogenesis in the species in which it normally exists, and for normal differentiation in other species?

7. What is (are) the significant phenotypic expression(s) of transformation which characterize a cell as malignant? These have thus far eluded precise definition. Many biological, biochemical, and morphological alterations accompany transformation; which are primary, which secondary, which significant, and which of little importance are questions not yet satisfactorily resolved. These questions absorb numerous investigators because identification of the significant expressions of transformation may suggest fresh approaches to problems in detection, diagnosis, prevention, and treatment of cancer.
8. Continued efforts to counteract or suppress the malignant properties of transformed cells so that they are once again subject to normal growth control, e.g., by chemicals - natural or synthetic - that induce differentiation.
9. Regulation of gene expression using viruses as tools.

OTHER ACTIVITIES

The Viral Oncology program supports workshops and conferences in its specific and related areas of concern. Those of particular interest in 1975 were investigator-initiated and consisted of the following: (1) "Workshops in Techniques in Cancer Research" at Aspen, Colorado, covering workshops and seminars on various aspects of cancer biology, carcinogenesis and clinical subjects; (2) five workshops at Cold Spring Harbor covering Animal Cell Culture, Molecular Cytogenetics, Animal Viruses, Yeast Genetics, and Immunogenetics; (3) a Gordon Research Conference on Cancer at Colby College, and (4) separate conferences on Cell Motility, RNA Polymerases, RNA Tumor Viruses, SV40, Polyoma Virus, and Adenoviruses, all at Cold Spring Harbor.

The Program Director (1) participates in various activities of the collaborative Virus Cancer Program such as in-house and advisory committees and project site visits. This interaction is useful in facilitating exchange of information in management of areas of mutual or overlapping interests and responsibilities; (2) participates in the deliberations and decision-making process as a member of the Molecular Control Working Group; (3) coordinates and organizes conferences and workshops on metastasis in connection with the U.S.-Japan Cooperative Cancer Research Program of the NCI; (4) assists in evaluating potential program administrators for the National Cancer Institute and other units of the National Institutes of Health as a member of a Civil Service panel.

NATIONAL ORGAN SITE PROGRAMS BRANCH

David L. Joftes, Ph.D., Chief

HISTORY AND CONTENT OF PROGRAM

The National Organ Site Programs consist of grant-supported national projects of targeted cancer research. Each national project is a planned and integrated research effort oriented toward cancer of a specific organ site. The planning, direction, coordination, and scientific administration of each national project are conducted at a headquarters institution other than the National Cancer Institute. The National Project Director, who is not an employee of the National Cancer Institute, is assisted in the planning and in the scientific administration of the national project by a Working Cadre of active research scientists from institutions throughout the nation.

The National Cancer Advisory Board (NCAB), after reviewing comprehensive plans for national programs of research, recommended the implementation of four national organ site projects. In March 1972, the Board recommended initiating the National Bladder Cancer Project under the direction of Dr. Gilbert H. Friedell, St. Vincent Hospital, Worcester, Massachusetts; in June 1972, the National Large Bowel Cancer Project under the direction of Dr. Murray M. Copeland, M. D. Anderson Hospital and Tumor Institute, Houston, Texas; in November 1972, the National Prostatic Cancer Project under the direction of Dr. Gerald P. Murphy, Roswell Park Memorial Institute, Buffalo, New York; and in November 1974, the National Pancreatic Cancer Project under the direction of Dr. Isidore Cohn, Jr., Louisiana State University School of Medicine, New Orleans, Louisiana. In March 1974, the Board approved support for developing information on the state-of-the-art and research opportunities in head and neck cancer under the direction of Dr. Joseph H. Ogura, Washington University School of Medicine, St. Louis, Missouri. A group of experts have held three major planning meetings and a Workshop on Head and Neck Cancer was held on February 16-18, 1976, in New Orleans, Louisiana. It is expected that the group will submit an application for the establishment of a National Head and Neck Cancer Project within a few months. Each of the four active projects is described below as an individual entity since each project is a substantial component of the Branch's activity. There are a total of 186 grants supported by the National Organ Site Programs at a cost of $14,100,000 (including the four headquarters grants at $1,229,298 including indirect costs).

NATIONAL BLADDER CANCER PROJECT

Olga G. Joly, D.D.S., Sc.D., Program Director

This project is supporting 55 grants at an estimated cost of $4,220,000 (including indirect costs and the headquarters grant of $290,020).

RESEARCH ACCOMPLISHMENTS

Development of Techniques to Identify Early Bladder Cancer Lesions: One of the goals of the National Bladder Cancer Project is to detect which individuals within a high risk group will develop tumors. Studies of bladder tumors in rats fed FANFT (N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide) show that at the end of ten weeks of carcinogen feeding, lesions become irreversible. At that same time, the surfaces of exfoliated bladder cells in the urine, examined with scanning electron microscopy, were found to be covered with pleomorphic microvilli instead of microridges or uniform microvilli found on normal cell surfaces (Jacobs et al., 1975). Examination of exfoliated bladder cells in the urine appears to provide a method of detecting early, noninvasive low grade lesions in this system, but the specificity of this finding remains to be determined.

Development of a Bladder Cancer Model for Evaluating Chemotherapeutic Regimens: The effect of single and combination chemotherapy was evaluated for antitumor activity against FANFT-induced bladder carcinoma in syngeneic mice. The single agents evaluated were cyclophosphamide, cis-diaminedichloro platinum (cis-PT-II), dactinomycin and adriamycin. Cyclophosphamide and dactinomycin were the most effective drugs in reducing tumor size, as reflected by total bladder weight. Combinations of chemotherapeutic agents were more effective than single agents. Mice receiving the combination of cyclophosphamide and 5-fluorouracil or adriamycin had the greatest reduction in bladder weight. There was also a high number of animals free of tumors among the treated mice when compared with the carcinogen-fed controls receiving no treatment (Soloway, 1975). Previous findings had shown that tumor cells would implant and grow better on inflamed bladder urothelium than on normal bladder epithelium. Intravesical chemotherapy with thiotepa, or epipodophyllotoxin VM-26 reduced the percent of implanted tumors (Weldon and Soloway, 1975). Intravesical adriamycin, bleomycin, and cis-PT-II had no significant effect. Systemic chemotherapy with adriamycin was effective in reducing the number of tumors, but cyclophosphamide yielded the better results (Soloway and Martino, 1976). These studies have encouraged urologists and oncologists to reconsider the available data on the chemotherapy of bladder tumors and have stimulated new clinical studies. Preliminary data from clinical studies appear to support, in general, the findings observed in the murine bladder tumor model.

REFERENCES

Jacobs, J.B., Cohen, S.M., Arai, M. and Friedell, G.H. "Scanning Electron Microscopy (SEM) Observations of Urinary Cytology from Rats Fed N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT)," Proc. Amer. Assoc. Cancer Res. 16:49, 1975. (CA-15945)

Soloway, M.S. "Single and Combination Chemotherapy for Primary Murine Bladder Cancer," Cancer 36:333-340, 1975. (CA-18643)

Soloway, M.S. and Martino, C. "Prophylaxis of Bladder Tumor Implantation," Urology, 7:29-34, 1976. (CA-18643)

Weldon, T.E. and Soloway, M.S. "Susceptibility of Urothelium to Neoplastic Cellular Implantation," Urology, 5:824-827, 1975. (CA-18643)

PROJECTIONS

New techniques for the detection and diagnosis of bladder lesions and improved utilization of existing methodologies are making it possible to better select appropriate treatment on an individual basis. Through the better utilization of urine cytology techniques, malignant lesions of the bladder are being detected which are not yet discernible by other techniques. These findings may make possible a test of aggressive treatment with adjuvant chemo- or immunotherapy in patients with very early disease.

Experimental studies in animal models indicate that scanning electron microscopic studies of exfoliated cells may improve our ability to detect bladder cancer in its earliest stages. If the prognostic significance of pleomorphic microvilli on exfoliated bladder cells in animal models proves to be specific and applicable to humans, the number of individuals identifiable as having bladder cancer before grossly visible lesions are observed would be expected to increase.

There is a major multidisciplinary study being conducted at four hospitals associated with UCLA to determine the effectiveness of non-specific immunotherapy in patients with advanced bladder carcinoma, and to monitor the immunological competence of such patients. The number of institutions participating under the National Bladder Cancer Collaborative Group A has increased. This is a joint effort in which the coordinating center, the central statistical center, the central laboratory, and all participating institutions are supported by separate grants. Four protocols are included in the "Evaluation of Management of Patients with Bladder Carcinoma": (1) the surveillance of patients with bladder cancer, (2) a study of their apparently normal bladder urothelium, (3) a clinical trial designed to assess the ablative and prophylactic effect of thiotepa instillations, and (4) clinical trial to compare the effectiveness of definitive radiotherapy with that of radiotherapy followed by appropriate surgery. In the evaluation of treatment, consideration is given not only to the length of survival but also to the quality of survival.

At one of the centers in the Collaborative Group A, selected biopsies are repeated at each follow-up examination to study the hypothesis that there is a definitive relationship between atypia and subsequent recurrences. Initial results from related experiments have indicated that the presence of atypia in the epithelium removed with the initial tumor biopsy is associated with a high rate of tumor recurrence and frequently with progression of the tumor to more invasive stages of the disease.

A new effort in chemotherapy has been initiated at two institutions to evaluate the potential of new drugs on bladder neoplasms; two more institutions are in the process of joining the chemotherapy group.

OTHER ACTIVITIES

Meetings:

1. NBCP Investigators' Workshop - January 25-27, 1976.
2. Urinary Bladder Epithelium Workshop - November 11-19, 1975.

Coordination:
Meetings for the purpose of exchange of information and coordination have been held with:
1. Dr. Gregory O'Conor (International Relations Office).
2. Dr. Ronald Herberman (Immunology, DCBD).
3. Ms. Jackie Parkman (NCI Office for Program Planning and Analysis).

Other Activities:
1. A procedures manual, "Assay for Tumor Specific Cytotoxicity," was supported by NBCP Headquarters through contract with Dr. Carol O'Toole, UCLA.
2. Headquarters personnel, grantees, and Dr. Olga Joly of NCI staff participated in the U.S.-Japan Meeting on Bladder Cancer Carcinogenesis in Hawaii, December 6-9, 1975.
3. Planning for a conference on Bladder Cancer to be held November 28-December 1, 1976 is well underway.

NATIONAL LARGE BOWEL CANCER PROJECT

Andrew Chiarodo, Ph.D., Program Director

There are 70 grants supported under the National Large Bowel Cancer Project for a total of $5,765,000 (including indirect costs and the headquarters grant of $340,799).

RESEARCH ACCOMPLISHMENTS

Carcinogenesis and Epidemiology:
Grantees of the National Large Bowel Cancer Project (NLBCP) have developed animal models of large bowel cancer in mice, rats, guinea pigs, and hamsters. These experimental cancers have been produced by administering several different carcinogens: dimethylhydrazine (DMH), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), methylazoxymethanol acetate (MAM), and methylcholanthrene.

In one study concerning inhibition of neoplasia of the large bowel (Wattenberg, 1975), it was found that the addition of two antioxidants, disulfiram and sodium diethylthiocarbamate, to the diet of mice receiving dimethylhydrazine prevented the development of colon cancer, while 90% of the controls developed an average of four colon cancers. Disulfiram did not inhibit the development of large bowel cancers in mice treated with MAM (Borchert and Wattenberg, in press). Other antioxidants which are poorly absorbed are being tested for inhibitory effects on MAM carcinogenesis in mice.

Molecular Control and Molecular Genetics:
Grantees have reported continued progress in defining molecular controls, cell kinetics, membranes, enzymes, and pyrimidine biosynthesis of colon cancer cells and in colon mucosa of animals under treatment with colon carcinogens. Procedures have been developed for the separation of particular classes of nuclei from colon mucosa of rats and humans. Nuclei recovered from different layers of density gradients have been compared for their contents of DNA, RNA,

histones, and non-histone proteins. Demonstrated differences in the total protein/DNA ratio are due to changing proportions of individual non-histone proteins. Both normal and tumor nuclei alter their non-histone protein complements during differentiation (Boffa et al., in press a,b). Nuclei from rat tumors induced by DMH were demonstrated to have a greater DNA synthesizing capacity than that of corresponding nuclei from control animals. Nuclear non-histone proteins were also found to differ from those of normal colonic mucosa. Striking increases were observed in the concentration of two classes of proteins with molecular weights of 44,000 and 62,000 in the tumor cell nuclei. A significant increase in these two non-histone nuclear proteins is observed three weeks after administering DMH and further exposure to the carcinogen causes a corresponding increase to even higher levels. Human adenocarcinomas of the colon were also found to have significant concentrations of non-histone nuclear proteins with molecular weights of 44,000 and 62,000.

Both histone and non-histone proteins are modified by (methyl-^{3}H) DMH. Non-histone nuclear proteins do not normally contain measurable amounts of mono- or di-methyl lysines but do contain methylarginine. Both methyl-arginine and methyl-lysines are detected in the non-histone proteins of rats given (methyl-^{3}H) DMH. Thus, the changes induced by alkylating agents such as DMH affect those amino acids (lysine and arginine) which are most likely to be involved in DNA binding. The investigator notes that to the extent that these DMH modifications alter the affinity of nuclear proteins for DNA, they are likely to initiate irreversible alterations in structure and function of the chromatin.

Immunobiology:
In rat models, three types of antigens have been demonstrated: the antigens of colon cancer, the gut-specific antigen, and widespread embryonal antigens. All of these have been demonstrated in solubilized preparations from tumor tissue. Rats immunized against various syngeneic colorectal carcinomas or fetal tissues were studied for their capacity to take transplants of colon cancer. While immunization against the tumor antigens of colon carcinoma caused high levels of isograft resistance, immunization of the recipients with colorectal carcinomas different from the test tumor or with various fetal tissues induced a reproducible but low level of resistance to isografting (Sjogren and Steele, 1975; Steele et al., 1975 a, b).

Clinical Cancer Treatment Research:
Grantees of the NLBCP are investigating the effectiveness of adjuvant immunotherapy with Bacillus Calmette-Guerin (BCG) and immune RNA in patients who, at operation, were found to have Dukes' B[2] and Dukes' C lesions. In one study (Mavligit et al., 1975), a total of 89 patients have been entered into the category of Dukes' C classification and are under investigation. It appears that the protection conferred by BCG alone may be slightly better than the protection conferred by the combination of 5-fluorouracil (5-FU) + BCG, at least in terms of disease-free interval. It is also interesting to note that 50 percent of the control patients who did not receive any adjuvant therapy following surgery exhibited a clinical relapse at 21 months, whereas only 25 percent who received BCG therapy alone following surgery had relapse at 21 months. There have been two deaths among the 33 patients who received BCG alone. Among the 50 patients who received a combination 5-FU + BCG, there have been no deaths. The survival curves of the groups which received either

BCG or the combination of 5-FU + BCG are better than those of the historical controls. The adjuvant effect of both BCG and 5-FU + BCG was more pronounced in those with more than six positive lymph nodes than in the group which had five or fewer positive lymph nodes. Among the 14 failures, half had local recurrences and half had distant metastasis. The 14 patients who had recurrences despite adjuvant therapy were found to be adequately immunocompetent at the time of relapse.

REFERENCES

Boffa, L.C., Vidali, G. and Allfrey, V.G. "Changes in Nuclear Non-Histone Protein Composition During Normal Differentiation and Carcinogenesis of Intestinal Epithelial Cells," Exptl. Cell Res. in press, a. (CA-14908)

Boffa, L.C., Vidali, G. and Allfrey, V.G. "Selective Synthesis and Accumulation of Nuclear Non-Histone Proteins During Carcinogenesis of the Colon Induced by 1,2-Dimethylhydrazine," Cancer in press, b. (CA-14908)

Borchert, P. and Wattenberg, L.W. "Inhibition of Macromolecular Binding of Benzo(a)pyrene and the Inhibition of Neoplasia by Disulfiram in the Mouse Forestomach," J. Natl. Cancer Inst. in press. (CA-15638)

Mavligit, G.M., Gutterman, J.U., Burgess, M.A., Khankhanian, N., Seibert, G.B., Speer, J.F., Reed, R.C., Jubert, A.V., Martin, R.C., McBride, C.M., Copeland, E.M., Gehan, E.A. and Hersh, E.M. "Adjuvant Immunotherapy and Chemo-Immunotherapy in Colorectal Cancer of the Dukes' C Classification, Preliminary Clinical Results," Cancer 36:2421-2427, 1975. (CA-15458)

Sjogren, H.O. and Steele, G., Jr. "Colon Carcinoma Antigens in the Rat," Ann. N.Y. Acad. Sci. 259:404-410, 1975. (CA-14924)

Steele, G., Jr., Sjogren, H.O. and Price, M.R. "Tumor-Associated and Embryonic Antigens in Soluble Fractions of a Chemically Induced Rat Colon Carcinoma," Int. J. Cancer 16:33-51, 1975. (CA-14924)

Steele, G., Jr., Sjogren, H.O., Rosenberg, J.E., Lindstrom, C., Larsson, A. and Leandoer, L. "Sequential Studies of Serum Blocking Activity in Rats Bearing Chemically Induced Primary Bowel Tumors," J. Natl. Cancer Inst. 54:959-967, 1975. (CA-14924)

Wattenberg, L.W. "Inhibition of Dimethylhydrazine-Induced Neoplasia of the Large Intestine by Disulfiram," J. Natl. Cancer Inst. 54:1005-1006, 1975. (CA-15638)

PROJECTIONS

Primary and transplantable colon cancers developed in animal models are being used by experimental pharmacologists studying the actions of new chemotherapeutic agents; by immunobiologists in studies on the immunobiology and immunotherapy of large bowel cancers; and by molecular biologists studying cell kinetics and molecular controls of such cancers.

Some of the studies in progress suggest biochemical targets that might be exploited therapeutically and others that may prove to be markers of colon cancer and/or antecedent lesions. Studies of normal mouse colon mucosa, DMH-induced mouse colon cancers, and of a human colon cancer cell line demonstrated a marked increase in activity of carbamyl phosphate synthetase in human and mouse colon cancers over that found in normal mouse colon cells. The enzyme activity was not inhibited by its end product, UTP.

N-(phosphonacetyl)-L-aspartate (PALA), a transition state analog for aspartate transcarbamylase (ATC), when injected into intact mice caused a prompt decrease in intestinal ATC activity. PALA not only inhibited ATC activity in cultured colon cancer cells, but it also inhibited the growth of these cells. The investigator is studying the effects of PALA on the growth of a transplantable colon cancer in mice and on the growth of a xenograft of a human colon cancer in hamster cheek pouches.

Grantees are reporting progress in defining individuals at risk and in developing and testing markers which might be of value in the early detection of large bowel cancer and of precancerous lesions of the large bowel. Markers which may be of value in identifying individuals carrying the gene(s) of heritable polyposis have been identified. Biochemical and biological markers of large bowel cancer are under investigation. Some of these markers have been demonstrated to have utility in the diagnosis of curable lesions.

The availability of animal models of large bowel cancer (primary and transplantable tumors) and of cultured cell lines of human and rodent colon cancers has made it possible for project grantees to investigate the effectiveness of established and new chemotherapeutic agents on the growth and metabolism of large bowel cancer. Drug combinations are being investigated for their effects on well established lines of large bowel cancer in vivo and in vitro. The effectiveness of post surgical adjuvant chemotherapy is also being studied in animals bearing transplantable colonic cancers.

In addition, three grantees working in cell kinetics and molecular control of cell proliferation and differentiation and molecular genetics are studying the actions of new and established chemotherapeutic agents on growth and certain biochemical characteristics of large bowel cancer.

The availability of cultured colon cancer cell lines, xenografts of human colon cancers, and of primary and transplantable colon cancers in rats and mice has provided grantees of the National Large Bowel Cancer Project with models to investigate the immunobiology of large bowel cancer, and from which to isolate new antigens, some of which appear to be colon cancer-specific, and some of which are capable of inducing a state in hamsters which results in

rejection of colon cancer xenografts. The chemical determinants of CEA are under investigation.

One investigator is developing a prosthetic sphincter for intestinal stomas. Another is investigating a new approach to liver perfusion with chemotherapeutic drugs.

OTHER ACTIVITIES

Meetings:
During the current year, two mini-workshops on Large Bowel Cancer were held:
1. The Application of Recent Investigations Toward the Control of Large Bowel Cancer - February 5-6, 1976.
2. New Approaches to Therapy of Large Bowel Cancer - March 6-7, 1976.

Both of these mini-workshops were multidisciplinary and succeeded in promoting communication between basic scientists and clinical investigators. The workshop participants with expertise in molecular biology, biochemistry, immunobiology, genetics, pharmacology, chemotherapy, gastroenterology, surgery, or clinical investigation recommended new programs of research designed to apply results of recent studies on the biochemistry and biology of large bowel cancer toward the development of control methods and the treatment of large bowel cancer, and to apply clinical observations toward expanded basic studies on the biology of large bowel cancer.

Coordination:
The NCI Program Director for the National Large Bowel Cancer Project, Dr. Andrew Chiarodo, represents the Project on the Gastrointestinal Tumor Study Group of DCT, which met three times last year.

Other Activities:
1. In December 1975, Cancer published a Conference Supplement of 30 papers that were presented before the Second Workshop on Large Bowel Cancer, which was held in Philadelphia, Pennsylvania, on January 23-24, 1975.
2. Two grantees of the National Large Bowel Cancer Project, Dr. Weisburger and Dr. Lipkin; the Project Director, Dr. Copeland; and the then Program Director, Dr. Joftes, participated in an international workshop in Geneva, Switzerland, in May 1975. The deliberations of the workshop were published as the International Union Against Cancer Technical Report Series Volume 19, titled "Colo-Rectal Cancer, Workshops on the Biology of Human Cancer, Report No. 2," Geneva, 1975.

NATIONAL PROSTATIC CANCER PROJECT

Andrew Chiarodo, Ph.D., Program Director

This project is currently supporting 44 grants at a total cost of $3,052,000 (including indirect costs and the headquarters grant of $364,759).

RESEARCH ACCOMPLISHMENTS

Treatment:
Randomized clinical trials of single chemotherapeutic agents for prostatic cancer are showing significant progress. The first studies have been directed at those patients who are in a far-advanced stage of disease and fail to respond to conventional endocrine methods of treatment, or for whom no other treatment is available. Using cytoxan or 5-FU, statistically significant improvement has been reported in patient survival, relief of pain, improvement in blood chemistries, tumor regression, and improvement in general well-being as measured by specific means. The trials are taking place at five institutions.

Etiology and Prevention:
Large differences in incidence or mortality rates for prostatic cancer have been reported for a number of populations. In comparing Blacks in Nigeria (low risk group) with Blacks in Washington, D.C. (high risk group), a 30 percent incidence has been reported in the high risk group as compared to 9 percent in the low risk group. The disease is first diagnosed at a later stage in Nigeria, and hormonal levels differ between these two populations as well as between cases and controls.

REFERENCES

Jackson, M.A. "Characterization of Prostatic Carcinoma Among Blacks: A Preliminary Report," Cancer Chemotherapy Reports 59:3-15, 1975. (CA-15448)

Scott, W.W., Johnson, D.E., Schmidt, J.D., Gibbons, R.P., Prout, G.P., Joiner, J., Saroff, J. and Murphy, G.P. "Chemotherapy of Advanced Prostatic Carcinoma with Cyclophosphamide or 5-Fluorouracil: Results of First National Randomized Study," J. Urology 114:909-911, 1975. (CA-15407, CA-15421, CA-15107, CA-15284, CA-15108, CA-14716)

PROJECTIONS

Treatment:
Studies are being directed at treatment of patients in earlier stages of disease, and toward combination chemotherapy with drugs of proven effectiveness in advanced stages of disease. Drug development studies specifically directed at prostatic cancer are promising. This area is significant because no other agency or group is directing its attention to this problem. Studies directed at determining the specific efficacy of radiotherapy and studies of agents or sensitizers that may increase the sensitivity of prostatic tumors to lower doses of radiation appear to have good potential.

Etiology and Prevention:
Epidemiologic studies are attempting to identify etiologic roles of dietary, socio-economic, occupational, sexual, and medical factors. Additional new projects for the development of experimental models for the study of prostatic cancer are in progress. In this area, studies are being conducted *in vitro* on the culture and propagation of normal and malignant prostate cells. This effort is necessary to determine whether viruses are a significant etiologic factor in this cancer. Although viral particles have been demonstrated in prostatic tissue, further studies are required to determine the nature of these particles and their relationship to the origin of human prostatic cancer. In addition, there has been some success in propagating new tumor models in rats through successive generations. These can be used to screen new chemotherapy agents for use in man.

Detection/Diagnosis:
Major efforts in this area have been and are directed to immunologically detecting prostatic cancer at its earliest onset. A significant number of cases throughout this country are now seen at a far-advanced stage, when very little can be done for the patient. Studies are in progress to develop more specific and sensitive assays, and preliminary results hold promise of improving our ability to detect this disease at an earlier stage of its progression. The same studies may help improve the monitoring of the efficacy of treatment.

In addition, studies are in progress to establish, on a morphological basis, criteria for detecting or discriminating borderline cases, and to develop better grading criteria for the diagnosis and prognosis of prostatic cancer.

OTHER ACTIVITIES

Workshops:
1. Evaluation of progress in the measurement of prostatic specific antigens for the detection and diagnosis of prostatic cancer.
   December 15, 1975 - Detroit, Michigan.
2. Problems in cell and organ culture of the prostate.
   December 19, 1975 - Buffalo, New York
3. Criteria of response for clinical trials.
   January 8, 1976 - New York, New York

Working Cadre Subcommittee Meetings for Updating Approved Plan:
1. Etiology/Prevention: September 16, 1975 - Buffalo, New York
2. Detection/Diagnosis: September 23, 1975 - Chicago, Illinois
3. Treatment: November 15, 1975 - Bethesda, Maryland

NATIONAL PANCREATIC CANCER PROJECT

David L. Joftes, Ph.D., Program Director

This project is in the process of funding its 17th grant for an estimated total of $1,062,891 (including indirect costs and the headquarters grant of $233,720).

RESEARCH ACCOMPLISHMENTS

None of the grants funded has been functioning long enough to have submitted a progress report.

PROJECTIONS

Research grants now getting underway involve tissue culture of bovine pancreas for use in carcinogenesis work; pancreatic metabolism of selected carcinogens; improvements in techniques of radiological diagnosis, scanning, and better use of ultrasonography; immunobiology and immunodiagnosis; etiology; biochemistry of pancreas cell differentiation; endocrinology; and pathology. The National Pancreatic Cancer Project continues to solicit additional applications for research in fundamental biology, etiology, epidemiology, detection and diagnosis, surgery, radiotherapy, pathology, immunobiology, immunoprophylaxis, immunotherapy, multimodality treatment, and supportive care.

OTHER ACTIVITIES

The National Pancreatic Cancer Project Working Cadre met four times during 1975 to review grant applications and continue updating its National Plan.

The NCI Program Director for the National Pancreatic Cancer Project, Dr. David L. Joftes, represents the project on the Gastrointestinal Tumor Study Group of DCT, which met three times last year.

During 1975, meetings were held with staff of DCT and DCBD to facilitate coordination and communication between our Divisions. Meetings will be held in the future with staff of DCCR and DCCP.

Staff of the National Organ Site Programs Branch developed an informational booklet, "Program Description, National Organ Site Programs," for distribution to potential applicants and other interested parties.

CLINICAL CANCER EDUCATION PROGRAM

Margaret H. Edwards, M.D., Chief

HISTORY AND CONTENT OF PROGRAM

The Clinical Cancer Education Program was initiated in July 1974 to replace the Clinical Cancer Training Program, which had been phased out by Executive order in January 1973 together with all other training programs at the National Institutes of Health. The objectives of the Clinical Cancer Education Program are similar to those of the earlier program; namely, to stimulate and expand multidisciplinary efforts in cancer education and teaching at the undergraduate, graduate, and continuing education levels. This will encourage students and practitioners dealing most directly with cancer to acquire and to apply basic knowledge and preventive, diagnostic, and therapeutic skills and experience to the problems of cancer and to the care of cancer patients.

The Clinical Cancer Training Program provided stipends for undergraduate and graduate trainees; the new Program contains no such provisions. Since 1966, when the Clinical Cancer Training grants were initiated, 4,274 individuals received support for periods of training ranging from three months for undergraduate medical and dental students to two-three years for graduate clinical trainees, in a wide variety of clinical specialties related to cancer, but chiefly in medical oncology, surgical and gynecologic oncology, pediatric oncology, cancer pathology, and radiology-radiotherapy. Graduate students constituted approximately 40 percent of the total number of trainees. Funds from both the earlier program and the current grants also provide support for selected faculty and staff, teaching equipment and supplies, consultants, and travel.

The budget for the Clinical Cancer Training Program ranged from $3.136 million in FY 1966 to $7.384 million in FY 1974, totaling $54.186 million over a ten-year period (an average of $12,600 per trainee). Grants were awarded to a total of 112 medical and dental schools, cancer institutes, and selected major hospitals affiliated with medical schools during this period. There are 12 Clinical Cancer Training grants currently receiving continuing support in FY 1976 for a total of $1.723 million. These grants are supporting, by means of stipends, the training of approximately 250 undergraduate and graduate trainees.

The Clinical Cancer Training grants were reviewed by two technical review committees especially established for this purpose; one committee reviewed only applications from schools of dentistry. These committees were abolished in 1973. In 1974, a new 24-member committee, known as the Clinical Cancer Education Committee, was chartered to review applications for the new grants. Since January 1975, this Committee has reviewed 108 applications and made program site visits to 81 institutions.

The first Clinical Cancer Education grants were awarded in June 1975 to 51 institutions for a total budget of $5 million. Since then, 41 applications have been approved for a total budget of $3.168 million. The FY 1976

appropriation for this Program is $7.492 million, which will permit payment of 51 continuation grants at $5.293 million and 23 of the 41 approved new grants.

PROGRAM ACCOMPLISHMENTS

The benefits of support for educational programs cannot be judged by the same criteria used for scientific research because the goals differ. Improvements in the scope and quality of cancer instruction, in the extent of multidisciplinary cancer teaching activities, in the numbers of undergraduate and graduate students seeking specialized periods of cancer instruction, and, ultimately, improvements in the quality of care provided to cancer patients by students and practitioners receiving their training at institutions supported by Clinical Cancer Education grants, are difficult to measure. The numbers of trainees receiving specialized cancer training can no longer be documented by the numbers of stipends dispensed.

In subjective assessments of the impact of the earlier Clinical Cancer Training Program, both staff members of the National Cancer Institute and individuals and groups of health professionals particularly concerned with cancer teaching have concluded that the continuing emphasis upon oncology provided by both the old and present Programs has maintained a heightened interest in cancer education, and has helped to promote collective thinking and actions concerning many aspects of cancer at grantee institutions. Most of the new Clinical Cancer Education grantees had the former type of grant, and site visits to these institutions have been impressed with the extent to which the goals of the earlier program have been achieved, particularly in terms of curriculum revisions, cancer electives, and the development of specialized graduate training programs.

In order to arrive at a more objective means of assessing the quality of cancer education, one active grantee institution with a staff of individuals particularly qualified in the field of medical education is undertaking a special project to develop Guidelines for the Evaluation of Cancer Education Programs. Additional funds have been added to the grant for this purpose, and results of the study should be available shortly after the end of the current fiscal year. The program director, Dr. Richard Caplan, Associate Dean for Continuing Medical Education, University of Iowa Medical Center, supported by grant R25 CA 18017, has held a series of workshops on this subject at which experts in medical education, cancer, educational psychology, and program analysis have pooled their knowledge and experience to develop a manual which will be useful both to educators in the health professions and to those responsible for education program planning and analysis.

Three information items have been prepared during the past year. These include a document developed by the Clinical Cancer Education Committee entitled "Essentials of Cancer Education," and two publications on the Training Programs of the National Cancer Institute, which appeared in the October 1975 issues of Cancer Research and Journal of the National Cancer Institute.

PROJECTIONS

The Clinical Cancer Education Program can have national impact only if funds continue to be obligated for this purpose. Further, there is a need to provide additional support for the training of an increased number of clinical oncologists, particularly in the specialties of medical oncology, pediatric hematology-oncology, radiation therapy, gynecologic oncology, surgical oncology, and cancer epidemiology. In order to determine the need for these specialists more objectively, a contract was awarded to Geomet, Inc., Gaithersburg, Maryland (NO1-CB-64017), on December 12, 1975. This study will be based on the current and projected volume of cancers of the types most appropriate to their specialized expertise in order to determine finite needs in four of these specialties for which no such data were readily available. Guidelines for a new specialized cancer training program have been developed and await the results of this study for further consideration.

Additional projects which should be carried out in the immediate future include the development of criteria for cancer education in residency programs and in medical specialties other than those mentioned above, such as gastroenterology. A conference on specialized training in gastrointestinal oncology will be held in September-October, 1976. Additional expenditures of funds will be required to conduct these activities.

OTHER ACTIVITIES

Coordination is maintained with the Training Branch, the Resources Branch, Division of Cancer Control and Rehabilitation, and to a lesser extent with the Office of Cancer Communications of the National Cancer Institute. Active participation is maintained in the activities of the American Association for Cancer Education and the American Cancer Society. Consultants to the Program include, in addition to the members of the Clinical Cancer Education Committee, numerous clinical oncologists throughout the country who are experts in cancer education; 50 such individuals have provided services to this Program in the past year.

RESEARCH MANPOWER DEVELOPMENT BRANCH

Barney C. Lepovetsky, Ph.D., J.D., Chief

During FY 1975 $23,123,000 was awarded for training grants and fellowships, and $2,806,000 in Research Career and Research Career Development Awards. Of special interest was the $8,121,000 investment in the new Institutional Fellowship Program authorized by the National Research Service Act of 1974. One hundred eighty-nine applications for this kind of support were received in eight areas of research in need of additional scientific personnel: carcinogenesis, chemotherapy, drug development, epidemiology, immunology, radiation, tumor biology, and viral oncology. While 78 approved applications were funded, 50 highly meritorious ones could not be supported. Those programs that were funded were hampered somewhat in their early recruiting efforts by the widespread concern of potential trainees as to the ramifications of the new payback provisions. Fortunately, these worries gradually seemed to subside and in the final analysis may not have been a major obstacle to the universities' recruiting efforts.

Complementing the new institutional fellowship program, the National Research Service Awards individual fellowship program was also begun. Two hundred forty-seven awards in the eight areas mentioned above were made at a cost of more than $2,761,000. Unfortunately, many exceptionally good applications could not be funded for lack of money.

After the two-year hiatus in funding new national research training and fellowship programs, the National Research Service Awards grants were welcomed by everyone interested in assuring a continuance of excellence in research training programs. The welcome was dampened, however, by an apprehension that both the legislative and executive branches would be reluctant to provide sufficient funds on a long-term basis to insure stability in these programs. For instance, it is anticipated that no new training grants will be made this year and that there will, in fact, be a net reduction in the existing training programs of about 30 T01 projects.

Again this year, the National Institutes of Health's Division of Research Grants was unable to review institutional research fellowship applications. The National Cancer Institute rechartered the 40-member Temporary Cancer Institutional Fellowship Review Committee. In April, that Committee evaluated about 120 applications that had been received by the end of February 1976. Those recommended for funding by the National Cancer Advisory Board at its June 1976 meeting will be considered for funding in the next fiscal year.

In the immediate future the national cancer research effort faces not only generally reduced research training, but also the danger of being limited to training areas identified by personnel projections that may not be accurate or timely.

THE CENTERS AND TREATMENT PROGRAM

Simeon T. Cantril, M.D., Associate Director

HISTORY AND CONTENT OF PROGRAM

Since the early 1960's, the entity now known as the Centers and Treatment Program has supported research in all of the scientific areas which are now designated as major thrusts of the National Cancer Program: detection and diagnosis, cause and prevention, cancer treatment, and cancer biology. This research is funded through peer reviewed grants of several types: Individual Research Project Grants; Research Program Project Grants, which combine a number of related clinical or preclinical research endeavors carried out by a coordinated group of scientists; Exploratory Studies Grants, which support the planning and development of cancer research, treatment, and outreach capabilities of cancer centers; Research Facilities Construction Grants, which provide funding for construction or renovation of needed cancer facilities; and more recently, Cancer Center Support (Core) Grants, which support the central personnel, facilities, and services required for the establishment, coordination, operation, and development of a unified cancer centers program. All of these types of grants are available to support appropriate programs at comprehensive or noncomprehensive cancer centers, and are administered and monitored by staff of the Centers and Treatment Program. Cancer centers also receive a variety of support from the Biomedical Research Program of the DCRRC, as well as from other NCI Divisions.

During FY 1976, the Division of Cancer Research Resources and Centers was reorganized, with resulting changes for the Centers and Treatment Program, previously called the Cancer Centers Program. The Centers Branch and the Research Facilities Branch have continued to operate under the Office of the Associate Director for Centers and Treatment, while a third Branch, Diagnosis and Treatment, was added to the Program. This newly established Branch brought together some programs which were previously assigned to other Branches of the DCRRC. It now includes the Detection and Diagnosis, Clinical Oncology, Drug Development and Pharmacology, Radiation Therapy, and Radiation Biology and Physics Programs. The coordination of these three Branches through the Office of the Associate Director in one aspect reflects the existing and rapidly developing coordination at cancer centers themselves of programs in diagnosis, treatment, and research of a multidisciplinary nature designed to upgrade cancer care on a nationwide basis. The change also gives an appropriate organizational visibility to the importance and volume of clinical research which is being carried out in cancer centers.

PROGRAM ACCOMPLISHMENTS

In the period since 1971, the Centers and Treatment Program has continued to respond to the directives of the National Cancer Act and the Amendments of 1974 by promoting the growth, development, and coordination of cancer centers across the country which could advance scientific knowledge about neoplastic disease, speed its effective application to the treatment of patients, provide

the multidisciplinary expertise needed for training in cancer, and provide expert demonstration and education programs in cooperation with other health agencies in their areas. In pursuing this goal, the Associate Director for Centers and Treatment coordinates the administrative functions of the three Branches under his administration and maintains close cooperation with the Associate Director for Biological Research, the Clinical and Research Manpower Branches, and also with those offices in other NCI Divisions closely related to the Centers and Treatment Program, especially Cancer Control and Rehabilitation, the Communications Office, the NCI Program Planning and Analysis Office, and the Division of Cancer Treatment. Such coordination within the NCI has been intensified during FY 1976 in response to the accelerated development and increasing visibility at both national and local levels of the Cancer Centers. Regular meetings have been held with staff of the Division of Cancer Control and Rehabilitation. Special meetings have been conducted to produce greater coordination between the Centers and Treatment Program and the Review and Referral Branch of DCRRC, the review committees which handle grants to Cancer Centers, and the National Cancer Advisory Board, through its Subcommittee on Centers and Construction. Combined efforts have continued in the area of communications, especially with regard to Communication Offices in Cancer Centers, with the NCI Office of Cancer Communications and the Division of Cancer Control and Rehabilitation (DCCR). Similarly, joint efforts have been directed toward an effective system for collection of nationally compatible data on cancer through cooperation with the International Cancer Research Data Bank and the NCI Surveillance Epidemiology and End Results (SEER) Program.

In conjunction with the Subcommittee on Centers and Construction of the NCAB, three Board site visits were conducted during FY 1976 to evaluate institutions which had requested recognition as Comprehensive Cancer Center Programs. One of these institutions, Ohio State University, was subsequently recognized by the National Cancer Institute as the 18th Comprehensive Cancer Center. In addition, a nationwide panel of scientists with special expertise in cancer has been assembled and is currently involved in the second round of a study utilizing the Delphi Technique aimed at developing parameters to permit future evaluation of expanding centers for degree of comprehensiveness, and also the review of existing Comprehensive Cancer Center Programs in order to document and upgrade the quality of these Centers.

PROJECTIONS

One of the earliest tangible outcomes of the current emphasis upon coordinated approach to the improvement of Cancer Centers has been the revision of Guidelines for Cancer Center Support and Exploratory Study Grants, which were developed on the basis of very widespread input from all sectors of the NCI, as well as from many outside advisors. Work has also been underway and is expected to produce similar guideline revisions in the near future for Cancer Research Facilities Grants and Research Program Project Grants. Meetings for Cancer Center Directors are being planned in a coordinated fashion. It is expected that, in the future, these meetings will be held probably on a semi-annual basis, with agendas structured to represent topics of concern to all NCI Divisions and Offices, as well as to the various centers themselves.

An extensive study is underway to develop a planning process for program evaluation of the Centers and Treatment Program, which is also expected to lead to the formulation of a long-range plan for the Program as a whole. An examination and analysis of all relevant documents relating to the Program has been completed, and this has resulted in the identification of the major issues in need of resolution at the current time and of the several levels of policy-making inputs and reporting requirements for the Centers and Treatment Program.

The major concern of the Centers and Treatment Program continues to be the achievement of the best possible geographic and scientific balance of a variety of Cancer Centers which are able, because of the many kinds of support furnished to multidisciplinary teams of dedicated scientists and physicians, to create and disseminate scientific knowledge which will be of the utmost benefit to cancer patients. This goal calls for a complex ordering of priorities based upon a wide variety of considerations which must be given proper balance. This effort to coordinate all of the available resources in order to produce the best possible benefits for cancer patients is expected to continue in the future.

DIAGNOSIS AND TREATMENT BRANCH

Roger H. Halterman, M.D., Chief

HISTORY AND CONTENT OF PROGRAM

The Branch Chief for Diagnosis and Treatment reports to the Associate Director for Cancer Centers and Treatment, Division of Cancer Research Resources and Centers. When the Division was reorganized, several program areas previously administered through the Clinical Investigations Branch were included in the Diagnosis and Treatment Branch. In addition, the entire clinical research effort from the Cancer Centers Program was shifted to this Branch, along with the Pharmacology Program. For purposes of programmatic continuity, the Immunology Program was transferred out of the Branch. The Branch Chief directs and coordinates the operations of the following program areas: Pharmacology; Clinical Oncology; Radiation Biology, Therapy, and Development; Prevention, Detection, and Diagnosis; and the clinical programs in Cancer Centers.

The Diagnosis and Treatment Branch functions as the organizational focal point for grant-supported clinically oriented research programs. The current program areas of the Branch are: Clinical Oncology, including Supportive Care Research, Chemotherapy, Surgery, and Multimodality Therapy; Radiation, including Radiation Biology, Radiation Therapy, and Radiation Development; Diagnosis Research and Prevention; Pharmacology; and Clinical Research Activities in the Centers Program. Detailed reports for each of these programs follow.

PREVENTION, DETECTION, AND DIAGNOSTIC RESEARCH PROGRAM

Roger H. Halterman, M.D., Acting Program Director

This program, begun in the 1960's, is involved primarily in programs and projects related to the prevention, early detection, and diagnosis of cancer; to the application of such knowledge to populations known to be at high risk; and to the current dissemination of this knowledge to the broad field of health professionals. The program is interested in and concerned with the improvement of current techniques as well as the encouragement and development of new modalities. To accomplish major program objectives, the following areas are stressed: (1) Promoting the development of new resource facilities to aid in the establishment of prevention-oriented projects. This includes the development of mechanisms for identifying high-risk populations through detection of predisposing and/or commonly associated laboratory, clinical, and other factors through existing and new technology. (2) Evaluation and improvement of known diagnostic procedures by application to selected subgroups of the population. (3) Utilization of current leads in the field of biological science, instrumentation, and computer technology to develop new diagnostic procedures. Of particular importance in this area is the development of noninvasive diagnostic methods.

The program maintains a continuing survey and evaluation of the state of knowledge regarding populations known to be at high risk. This necessitates a close liaison with other programs of the National Cancer Institute and other Federal programs concerned with identifying potential carcinogenic agents, and monitoring individuals known to be at risk of exposure and ultimately at risk of developing a malignant disease. The program thus follows closely developments and accomplishments in advancing the prevention, detection, and ultimate treatment of human cancer. In addition, this program is concerned with identifying special needs and opportunities where increased or specialized manpower facilities and efforts might be productive. As new opportunities for advancement are identified, the program is responsible for developing activities in these areas and for providing continuing technical, administrative, and financial support. Special emphasis will be given to the development of regional resource capabilities to fulfill these program responsibilities.

CLINICAL ONCOLOGY PROGRAM

Roger H. Halterman, M.D., Acting Program Director

The Clinical Oncology Program now encompasses the activities of Supportive Care Research; single Modality Research in the areas of Chemotherapy, Surgery, Radiation Therapy; and the combined activities of all these disciplines into a coordinated effort. The program continues to provide scientific direction for grant-supported research involving the use of drugs, surgery, and radiation in the treatment of cancer. The four major thrusts of the program are: (1) clinical therapeutics, (2) developmental studies, (3) toxicity, and (4) cancer-related disorders. In addition to these primary treatment thrusts, the program has an area of supportive care research which is directed towards developing the necessary knowledge to permit long-term control of hemorrhage by repeated platelet transfusion and control of infection by leukocyte transfusions and protective environments.

For the first time, this program brings together into one unified and identifiable program all of the needed resources to carry out productive clinical research. The major emphasis for the entire Clinical Oncology Program is development of more efficient and improved techniques for both the diagnosis and treatment of human cancer. Other appropriate studies include the natural history of human cancer, the body's immune defense mechanisms, the elucidation of effects of various treatments and related tissue responses, and the importance of host factors in disease occurrence, rate of progression, curability, etc. Improved experimental design, data retrieval, and statistical analysis are also integral aspects of this program. Examples of studies supported in this area are: (1) Therapeutic studies of all types, alone or in combination, i.e. surgery, chemotherapy, radiation, immunotherapy, or combinations thereof. (2) Studies of therapeutic adjuvants of all types including protected environment, platelet transfusion, and preservation, etc.

RESEARCH ACCOMPLISHMENTS

The following are examples of recently published work supported by the Prevention, Detection, and Diagnosis Research Program and the Clinical Oncology Program.

X-rays of the pancreatic duct can now be obtained by a nonoperative endoscopic approach (endoscopic retrograde cholangiopancreatography, ERCP). White and Silverstein (1976) report, after more than two years' experience with this technique, that the pancreatic duct can be visualized in 85 to 90% of patients. The test can be used to detect pancreatic carcinoma in a symptomatic patient and to search for an operative pancreatic lesion in a patient with known recurrent or chronic pancreatitis. Stenosis or obstruction of the main pancreatic duct with or without proximal duct dilation are the characteristic abnormalities noted in pancreatic carcinoma. In patients with chronic pancreatitis it may be difficult to differentiate an inflammatory from a neoplastic stricture by either operative or endoscopic pancreatography. In the future, cytologic and biochemical examination of the pancreatic secretions obtained at ERCP may increase the accuracy of diagnosing carcinoma.

Urinary excretion of nonesterified cholesterol (NEC) in 170 women with cervical and endometrial carcinomas has been investigated (Acevedo et al., 1975). The results showed NEC hyperexcretion in 65 of 68 women with active carcinoma of the cervix, including 13 patients with carcinoma in situ, and in 42 of 45 women with active carcinoma of the endometrium. In contrast, NEC excretion was normal in all (77) patients with other malignant and/or benign diseases of the pelvic organs or with non-steroid-related neoplasms; in 39 of 48 patients with benign and/or malignant breast disease other than carcinoma; and in 101 of 111 patients with a variety of non-neoplastic diseases.

Sequential studies in patients with uterine carcinomas demonstrated an almost perfect correlation between NEC excretion and the clinical status of the patient following surgical and/or radiation therapy. It appears, therefore, that urinary NEC determinations can be used as an additional diagnostic biochemical test to detect active carcinoma of the steroid-producing glands and their main target organs, and that in women with uterine carcinomas, the test can be used as an objective laboratory method to monitor the course of the disease and the response of the patient to therapy.

As part of a program directed toward elucidation of the role of viruses in mouse and human breast cancer, a variety of immunological techniques were applied to a study of the humoral immune response of mice and of humans to their breast tumors (Bowen et al., 1976). Tumor-bearing mice were found to produce antibodies against a complex array of tumor cell-associated antigens. Tumor-free mice, whether of high or low mammary cancer incidence strains, were remarkably free of antibodies that could label mouse mammary tumor virus (MMTV) particles, although some sera contained antibodies to viral components.

Patients with breast cancer also had antibodies against a variety of antigens associated with their own and homologous breast cancer cells. These antibodies reacted with heterophile, embryonic, and other tumor-associated antigens, some of which appeared to be viral. Sera of some patients with breast cancer gave

positive immunofluorescence reactions with mouse mammary tumor cells grown in tissue culture and producing MMTV. Although human-mouse cross-reactions must be interpreted with caution, these data suggest that a virus putatively associated with human breast cancer is antigenically related to MMTV.

For the past several decades, the 5-year survival rates for patients with osteogenic sarcoma have remained virtually unchanged, ranging between 10 and 20%. A four-drug adjuvant regimen was begun in 1971 by the Southwest Oncology Group for treating patients with nonmetastatic osteogenic sarcoma. All patients had surgical amputation for the primary lesion, followed by cyclophosphamide, vincristine, melphalan, and adriamycin in defined combinations intermittently over a 72-week period. Of the 18 patients, 10 (55%) remain free of disease 24 months or longer from the time of amputation. In the comparison series (33 children treated by surgery alone between 1953 and 1972 at M.D. Anderson Hospital), the time to appearance of metastases ranged from 1 to 18 months, with the median at 4 months. In the latter series, 90% of the treatment failures among these patients had developed metastases within 12 months. Therefore, it seems unlikely that any of the 10 disease-free patients in this study will develop "late" metastases.

The use of high dose methotrexate (with citrovorum factor rescue) plus adriamycin as adjuvants immediately after surgery for metastatic osteogenic sarcoma has increased the 1- and 2-year disease-free survival rate to between 50 and 80%. In an attempt to avoid amputation of a weight-bearing limb, a program of preoperative chemotherapy followed by total femur and knee replacement with a vitallium prosthesis for osteogenic sarcoma of the distal femur was begun at Memorial Sloan-Kettering Cancer Center in 1973. Initial results of treatment in the first 20 patients entered in this program have recently been described (Rosen et al., 1976). Eighteen of the patients had clinically measurable primary tumors and 17 of these demonstrated a decrease in tumor size prior to surgery, while on chemotherapy. Twelve of these patients with osteogenic sarcoma of the distal femur have had total femur and knee joint replacement, and three patients with osteogenic sarcoma of the proximal tibia have had total knee replacement.

Although the first patient treated had extensive soft tissue and muscle resected at the time of surgery, she walks well with the aid of a cane and has full range of motion of the hip. Two patients entered into treatment with pulmonary metastases. In one, the metastases completely disappeared following initial chemotherapy prior to surgery. In the other, who had bilateral multiple small pulmonary metastases, the metastatic tumors did not appear to grow during the three months of preoperative chemotherapy. After total femur and knee replacement, the patient underwent bilateral thoracotomies and all of the nodules were completely removed; there was no evidence of viable tumor cells.

There has been no evidence of soft tissue recurrence in any of the 15 patients who have undergone surgery. The ultimate evaluation of this approach to treatment of primary osteogenic sarcoma awaits longer observation to determine limb function and the continued disease-free status, once adjuvant chemotherapy is discontinued.

Treatment with adriamycin has been limited to induction or consolidation therapy rather than long-term maintenance largely because of severe dose-dependent cardiomyopathy. Since adriamycin appears to be the best single agent for a number of malignant diseases, and in some types of cancer is the only drug of therapeutic significance, the clinical and pathologic features of adriamycin cardiomyopathy have been reviewed and risk factors determined (Minow et al., 1975). Two types of cardiac effects are attributed to adriamycin: (1) various nonspecific electrocardiogram abnormalities, and (2) dose-related congestive heart failure. Attempts are being made to detect adriamycin-induced cardiotoxicity before clinically significant damage has occured. In addition, several factors which increase the risk of cardiac toxicity developing have been identified: cardiomyopathy developed at lower cumulative doses of adriamycin in patients who had had prior radiotherapy in which any portion of the heart was included in the treatment field, and in patients who had received cyclophosphamide concurrently with adriamycin. Based on an analysis of the available data, recommendations for safe use of adriamycin have been established by the M.D. Anderson Hospital.

Pelvic exenteration consisting of hystovaginectomy, oophorectomy, cystectomy, and occasional colon resection is usually recommended as the treatment of choice for rhabdomyosarcoma of the vagina and uterus in children. This radical operation requires diversion of urine by ureteroileostomy or ureterosigmoidostomy and occasional colostomy. Planned coordinated management of rhabdomyosarcoma and pre- and postoperative chemotherapy and radiotherapy has made possible a less radical anterior exenteration in patients with vaginal and uterine involvement and morbidity and mortality associated with cystectomy and urinary diversion can now be avoided, without any apparent reduction in long-term tumor-free survival.

Kumar et al. (1976) report on three children whose rhabdomyosarcomas of the vagina or uterus were managed by modified radical resection combined with pre- and/or postoperative chemotherapy and high-dose irradiation. Two patients had complete tumor regressions following preoperative chemotherapy (vincristine, cyclophosphamide, and dactinomycin) and irradiation. The third patient received no preoperative therapy but was given postoperative radium implantation, irradiation, and chemotherapy. The surgical approach consisted of hystovaginectomy and oophorectomy without urinary diversion. These patients are free of tumor for 32, 44, and 54 months, respectively. There were no serious toxic reactions to the drugs, nor any significant postoperative urinary tract problems.

There has been a gratifying increase in the survival of children with cancer as a result of more effective methods of treatment that have evolved during recent years. Radiation and many of the drugs used to treat cancer are themselves carcinogenic under certain conditions, however. A study was therefore inaugurated in order to determine the late consequences of therapy (D'Angio et al., 1976). One hundred two long-term survivors of childhood cancers with second malignant neoplasms were collected from ten institutions. Forty-seven cases fulfilling study criteria were studied to determine the risk of developing a second malignancy in irradiated fields after exposure to various chemotherapeutic agents. The case-control method was used for these determinations.

The risk of developing such tumors was decreased by a factor of 7 in patients receiving actinomycin-D. The "protective" effect of this drug was more pronounced in patients receiving repeated courses of actinomycin. No change in relative risk was found for children given antifolates, the vinca alkaloids, or alkylating agents. Actinomycin-D "protection" was an unexpected finding because the drug is oncogenic in animals and enhances radiation, also a known oncogen. These results indicate the need for careful long-term observation of cancer survivors to determine the late effects of multimodal cancer treatment.

The risk of infection is especially high among patients with hematologic malignancies because bone marrow function is often compromised by the disease process as well as by cytotoxic therapy. Since serious infections can develop in these patients even while they are in protected environment units, records of 102 studies on 89 patients have been reviewed to determine the sites of infection and the etiologic agents (Bodey and Rodriguez, 1975). Major infections occurred during 22 studies. The majority of both minor and major infections originated during the first five weeks after the patients entered the protected environment units; most of the causative organisms had persisted in the patients after their entry into the units despite the use of prophylactic antibiotics. The majority of infections were cases of cellulitis, pharyngitis, pneumonia, and septicemia. Most of the infections were caused by Gram-negative bacilli. The frequency of infectious complication was inversely related to the circulating neutrophil count.

This study shows that, although the protected environment-prophylactic antibiotic program reduces the risk of infection, the distribution of infection by site and causative organism in patients in such a program is similar to that of other cancer patients. Further reduction in infection in cancer patients on the protected environment-prophylactic antibiotic program will require the use of new antibiotic regimens or the development of new approaches to antibiotic prophylaxis.

Bone marrow transplantation is emerging as a viable therapeutic approach to a number of usually fatal diseases. A successful transplantation program is a complex clinical effort requiring a team approach. At the University of California School of Medicine (UCLA), the team consists of clinicians from the Departments of Pediatrics and Internal Medicine, clinical pathologists, geneticists, basic scientists from the Departments of Medical Microbiology and Immunology, and experts in tissue typing from the Department of Surgery. The purposes of the bone marrow transplantation program are twofold: the application of a new therapeutic modality to the management of malignant disorders with poor prognosis; and research directed toward improving the efficacy, broadening the applicability, and understanding the long-term effects of marrow transplantation. To date, 30 transplants have been done at UCLA for severe aplastic anemia and acute leukemia. The results obtained with 16 patients have recently been reported (Cline et al., 1975); the additional 14 patients have been treated so recently that results can not yet be evaluated.

Patients are prepared for bone marrow transplantation by pretreatment with cytotoxic drugs and/or total body irradiation. They are also kept in strict isolation, bathed daily in betadine, and receive gastrointestinal antibiotic prophylaxis. After transplantation, as prophylaxis against graft-versus-host

disease, patients receive methotrexate. All post-transplant blood products are irradiated to inhibit engraftment with transfused lymphocytes.

Three of six patients treated for aplastic anemia have achieved complete or partial hematologic recovery. Of four patients treated for refractory acute lymphoblastic leukemia, three are still living (after intervals of 75 to 120 days) with no evidence of disease; a fourth died after 100 days, of interstitial pneumonitis, but with no evidence of leukemia. Two of six patients treated for refractory acute myeloblastic leukemia are alive 100+ and 150+ days after marrow grafting; neither has evidence of disease. An additional patient died after 47 days of renal and pulmonary failure, but had no evidence of leukemia at the time of death. Two patients died of recurrent disease and the sixth died only eight days after therapy, of severe intestinal toxicity.

Bone marrow transplantation is restricted to a small percentage of potential recipients because of histocompatibility barriers that cannot be overcome. The prevention and control of graft-versus-host disease are major challenges to extension of bone marrow transplantation. Two general approaches have been explored: removal of reacting cells, and suppression or impairment of undesired immunologic reactions. With greater appreciation of the mechanisms that reduce immune response and with better methods for removal of particular cell types, efforts to control and prevent graft-versus-host reactions will be extended, and barriers that currently prevent extensive use of bone marrow transplantation will be breached.

REFERENCES

Acevedo, H.F., Campbell, E.A., Frich, J.C., Jr., Merkow, L.P., Hayeslip, D. and Gilmore, J. "The Significance of the Excretion of Nonesterified Cholesterol in Patients with Uterine Carcinomas," Cancer 36:1459-1469, 1975. (CA-12092)

Bodey, G.P. and Rodriguez, V. "Infections in Cancer Patients on a Protected Environment-Prophylactic Antibiotic Program," Amer. J. of Med. 59:497-504, 1975. (CA-10042, CA-05831)

Bowen, J.M., Dmochowski, L., Miller, M.F., Priori, E.S., Seman, G., Dodson, M.L. and Maruyama, K. "Implication of Humoral Antibody in Mice and Human to Breast Tumor and Mammary Tumor Virus-associated Antigens," Cancer Res. 36:759-764, 1976. (CA-05831)

Cline, M.J., Gale, R.P., Stiehm, E.R., Opelz, G., Young, L.S., Feig, S.A. and Fahey, J.L. "Bone Marrow Transplantation in Man," Ann. Int. Med. 83:691-708, 1975. (CA-15688)

D'Angio, G.J., Meadows, A., Mike, Y., Harris, C., Evans, A., Jaffe, N., Newton, W., Schweisguth, O., Sutow, W. and Morris-Jones, P. "Decreased Risk of Radiation-Associated Second Malignant Neoplasms in Actinomycin-D-Treated Patients," Cancer 37:1177-1185, 1976. (CA-17657, CA-02501, CA-03713)

Kumar, A.P.M., Wrenn, E.L., Jr., Fleming, I.D., Hustu, H.O. and Pratt, C.B. "Combined Therapy to Prevent Complete Pelvic Exenteration for Rhabdomyosarcoma of the Vagina or Uterus," Cancer 37:118-122, 1976. (CA-08480, CA-08151)

Minow, R.A., Benjamin, R.S. and Gottlieb, J.A. "Adriamycin (NSC-123127) Cardiomyopathy - An Overview with Determination of Risk Factors," Cancer Chemother. Rep. 6:195-201, 1975. (CA-10379, CA-14528, CA-11520, CA-05831)

Rosen, G., Murphy, M.L., Huvos, A.G., Guttierrez, M. and Marcove, R.C. "Chemotherapy, En Bloc Resection, and Prosthetic Bone Replacement in the Treatment of Osteogenic Sarcoma," Cancer 37:1-11, 1976. (CA-08748, CA-05826)

Sutow, W.W., Sullivan, M.P., Fernbach, D.J., Cangir, A. and George, S.L. "Adjuvant Chemotherapy in Primary Treatment of Osteogenic Sarcoma," Cancer 36:1598-1602, 1975. (CA-02501, CA-03713, CA-12014, CA-03161, CA-07357, CA-12644)

White, T.T. and Silverstein, F.E. "Operative and Endoscopic Pancreatography in the Diagnosis of Pancreatic Cancer," Cancer 37:449-461, 1976. (CA-14380)

DRUG DEVELOPMENT AND PHARMACOLOGY PROGRAM

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HISTORY AND CONTENT OF PROGRAM

The Drug Development Program was initiated in 1966 as one of six units of the extramural activities program structure. It remained a part of the Biomedical Research Program of this Division until 1976, when it was transferred to the Diagnosis and Treatment Branch of the Centers and Treatment Program.

The workscope of the Drug Development Program concerns the development and evaluation of chemotherapeutic agents which act specifically or selectively against malignant growth with minimal toxicity to the host. The primary objective is to provide direction and encouragement to outstanding investigators in diverse scientific disciplines to pursue research in the chemical, pharmacological, and cytological aspects of abnormal as well as normal growth processes. The Program effort encompasses a broad range of research activities involving synthesis of new antitumor agents, experimental therapeutic test systems, pharmacological and toxicological evaluation in preclinical and clinical stages, and studies in cell kinetics, mechanisms of drug action and molecular transformations, particularly those related to the control of neoplastic processes.

During FY 1976, the Drug Development Program supported 394 Research Project grants, four Specialized Program Project grants, and one Core grant. The total monetary commitment was $25.76 million distributed as follows:

Synthesis and Isolation: (213 grants, $10.41 million, 40%)
Preparation of potential anticancer agents through synthetic chemical processes or by isolation from microbial, plant, or animal sources. Determination of structure and identification of known and potential chemotherapeutic compounds.

Preclinical Drug Evaluation: (84 grants, $5.05 million, 20%)
Screening tests for anticancer activity, experimental therapeutics and toxicologic and other pharmacologic studies, criteria for selection of safe and effective dose regimens, countermeasures for toxicity.

Mechanism of Drug Action: (94 grants, $6.44 million, 25%)
Physiological disposition and drug metabolism, biotransformations, mode of action relating to antitumor and pharmacologic responses. Basic studies for understanding of the mechanisms of growth inhibition; cell kinetics.

Clinical Pharmacology: (3 grants, $0.26 million, 1%)
Pharmacologic and toxicologic studies and experimental therapeutic investigations in humans through controlled clinical trials with selected drugs; correlation of preclinical and clinical data.

Research Program Projects and Core: (5 grants, $3.60 million, 14%)
Broad multidisciplinary projects or intensive effort for total drug evaluation including research in synthesis, experimental therapeutics, preclinical

toxicicology, pharmacology, mechanisms of drug action, and clinical pharmacology.

RESEARCH ACCOMPLISHMENTS

Solid Tumor Models for Assessment of Treatment Modalities:
A major limitation in devising successful chemotherapy of solid tumors is that only a fraction of all tumor cells are in an active proliferative stage. The kinetic parameters of such cells cannot be delineated unless their morphology is distinguished and studies are limited to biologic assays for clonogenicity. Three solid tumor models adapted from transplantable Morris hepatoma series serve to provide critical data on the effects of chemotherapeutic agents at four organizational levels: the molecular, cellular, organ, and organism levels. Detailed investigations (Looney et al., 1976) of tumor growth rates of rapid, intermediate and slow-growing type, actual volume doubling times, tumor cell cycles, and thymidine labeling indices have established that each specific solid tumor line possesses a unique set of biological characteristics which could serve as parameters for devising improved therapeutic schedules. Different modalities of treatment such as drugs administered individually or in sequence, drugs plus radiation, or drugs plus immunotherapy could be evaluated to yield quantitative data on the sequence of toxic effects related to therapy and the kinetics of recovery of the host and tumor. Laboratory studies on well-defined solid tumor models can yield quantitative data concerning the time sequence of host toxicity related to therapy and the kinetics of host and tumor cells. Studies on the correlation between animal models and the patient may provide improved regimens for management of solid tumors in humans.

A potentially useful screening model, the murine neuroblastoma C1300, has been developed (Finkelstein et al., 1975) for neuroblastoma in children. Histopathologic, electron microscopic, and chemical studies demonstrated the presence of catecholamines in this tumor. Its biological properties and enzyme content suggested that the C1300 tumor model possessed many specific biochemical features of human neuroblastoma. The cellular kinetics of the murine model, particularly its shorter DNA synthesis time, may explain the lack of activity of cell cycle-specific agents. It appears to be preferentially sensitive to mitotic phase-nonspecific drugs. A favorable response rate is also seen in children with metastatic neuroblastoma treated with a combination of two cell cycle-nonspecific drugs, cyclophosphamide and imidazole carboxamide, plus vincristine. The sensitivity of murine neuroblastoma to specific chemotherapeutic agents and its unique biologic characteristics emphasize the need for further evaluation of the C1300 as a laboratory model for corresponding neural neoplasms in the human.

Cyclic CMP (Cytidine 3'5'-monophosphate) - Isolation, Biological Function and Significance:
The presence of cyclic CMP was recently demonstrated in mammalian cells (Bloch, 1974). Specifically, the cyclic nucleotide was found in relatively high concentration in leukemia L1210 cells and in regenerating rat liver; the concentration in regenerating liver was approximately 100 times that in normal liver. Urine samples from untreated patients with leukemia contained significant quantities of cyclic CMP whereas the metabolite was absent from the urine of control

subjects. Cyclic CMP stimulated the proliferation of leukemia L1210 which was inhibited by cyclic AMP and cyclic GMP. These findings suggest a correlation between rapid cell proliferation and high cyclic CMP levels and provide a rationale for use of structural analogs for potential control of cell growth. The presence of detectable amounts of cyclic CMP exclusively in the urine of leukemic patients is also of interest as a basis for development of a possible diagnostic or prognostic test. The in vitro biosynthesis of cyclic CMP from CTP has recently been demonstrated (Bloch et al., 1976).

Biochemical Pharmacology and Mechanism of Action Studies on Selected Clinical Chemotherapeutic Agents:

N-Trifluoroacetyladriamycin-14-valerate (AD 32), a semisynthetic analog of adriamycin, exhibits significantly greater antitumor activity than does adriamycin or daunorubicin in two experimental mouse tumor systems under similar assay conditions (Israel et al., 1975). The compound inhibits the growth of cells in culture and produces a highly significant antitumor effect against two mouse leukemias in vivo. According to prevailing theory, the basic glycosidic amino group must be free for expression of biological activity by the antitumor anthracycline antibiotics. In AD 32 the amino group is no longer basic, having been converted to an amide. In view of this, the superior experimental therapeutic effect of AD 32 over adriamycin represents a unique observation. Toxicological advantage of AD 32 was evidenced by its optimal dose range, which is significantly greater than the lethal dose of adriamycin in mice, and by its lack of delayed toxicity. Therapeutically effective dose levels of AD 32 are 10 to 15 times greater than the optimal dose of adriamycin on a weight basis. The very high percentage of long-term survivors with high doses of AD 32 suggests that this analog does not produce delayed toxicity in the mouse. Myocardial degeneration, which is a limiting factor in the clinical use of adriamycin, was not seen with AD 32 in this study; further evaluation for possible cardiotoxicity is planned. Analogs of adriamycin with greater therapeutic efficacy and less toxicity than the parent antibiotic would be of interest from the standpoint of clinical treatment of leukemias, lymphomas, breast cancer, soft tissue sarcomas, and bone cancers.

Maytansine, a novel ansa macrolide isolated from various Maytenus species, is an antitumor agent that significantly inhibits mouse P-388 lymphocytic leukemia in dosages of micrograms per kilogram of body weight, and is active over a 50- to 100-fold dosage range. Maytansine also shows significant inhibitory activity against the L1210 mouse leukemia, the Lewis lung carcinoma, and the B-16 melanocarcinoma murine tumor systems. At present, Maytansine is undergoing Phase I study for its potential therapeutic and toxicologic effects in clinical trials. Maytansine irreversibly inhibits cell division in eggs of sea urchins and clams. It causes the disappearance of a mitotic apparatus or prevents one from forming if added at early stages (Remillard et al., 1975). Maytansine does not affect formation of the mitotic organizing center but does inhibit in vitro polymerization of tubulin. Although maytansine and vincristine inhibit in vitro polymerization of tubulin at about the same concentrations, vincristine is about 100 times less effective as an inhibitor of cleavage in marine eggs. Further detailed studies on the mechanism of action of this compound are in progress.

Potential Role of Ergot Alkaloids in Prevention and Therapy of Breast Cancer: Several ergot alkaloids and ergoline derivatives appear to mimic the activity of the hypothalamic-prolactin inhibitory factor (PIF). They appear to be relatively specific for prolactin and are efficacious inhibitors of the secretion of this hormone in mice, rats, domestic animals, and in the human as well. Administration of these drugs to rats bearing carcinogen-induced mammary carcinomas results in a prompt regression of tumors. Chronic treatment of a high breast cancer strain of mice with ergoline compounds virtually prevents the subsequent development of spontaneous breast cancers. Treated mice, totally free of palpable breast cancers, appear to be normal in every respect. From the results of these studies (Welsch, 1976) and others, it appears that prolactin plays a key role in murine breast carcinogenesis. An important question is whether or not prolactin has an influential role in the development of human breast cancers. A series of studies demonstrates that at least 15% of human breast cancers respond to the in vitro growth stimulatory effects (increased DNA synthesis) of prolactin. If prolactin can definitively be shown to be important in the development and growth of normal, precancerous or cancerous breast tissues in humans, as it is in rodents, then prophylaxis or chemotherapeutic control of the disease by appropriate drug-mediated hormone suppression may be feasible.

REFERENCES

Bloch, A., Dutschman, G. and Maue, R. "Cytidine 3',5'-Monophosphate (Cyclic CMP) II. Initiation of Leukemia L-1210 Cell Growth In Vitro," Biochem. & Biophys. Res. Comm. 59:955-959, 1974. (CA-12585)

Bloch, A. and Leonard. R.J. "Formation of Cytidine 3',5'-Monophosphate (Cyclic CMP) in Extracts of Leukemia L-1210 Cells," Proc. A.A.C.R. 17:203, 1976. (CA-12585)

Finkelstein, J.Z. and Weiner, J. "Cytokinetic Considerations of Murine Neuroblastoma (C1300) as a Screening Model for the Childhood Disease," European J. of Cancer 11:175-179, 1975. (CA-14658)

Israel, M., Modest, E.J. and Frei, E. "N-Trifluoroacetyladriamycin-14-valerate, An Analog with Greater Experimental Antitumor Activity and Less Toxicity than Adriamycin," Cancer Res. 35:1365-1368, 1975. (CA-19118)

Looney, W.B., Mayo, A.A., Kovacs, C.J., Hopkins, H.A., Simon, R. and Morris, H.P. "Solid Tumor Models for the Assessment of Different Treatment Modalities: II: Rapid, Intermediate, and Slow-Growing Transplantable Rat Hepatomas," Life Sciences 18:377-390, 1976. (CA-13102)

Remillard, S., Rebhun, L.I., Howie, G.A. and Kupchan, S.M. "Antimitotic Activity of the Potent Tumor Inhibitor Maytansine," Science 189:1002-1005, 1975. (CA-12059)

Welsch, C.W., Calaf de Iturri, G. and Brennan, M.J. "Potential of Ergot Alkaloids in Control of Breast Cancer," Cancer (in press). (CA-13777)

PROJECTIONS

This section outlines research in progress for clinical application or for a better understanding of the mechanism of action of new chemotherapeutic agents. The study of selectivity of drug action has long-range implications and is imaginative in its goals. The development of precise methods for identification of specific cell functions and kinetic aspects of cell proliferation promotes direct application to studies of the selective effects of anticancer agents.

Chronobiological Approach to Cancer Chemotherapy:
The existence of circadian fluctuation in host toxicity is well established from work in chronopharmacology. Responsiveness and sensitivity to drugs fluctuate significantly from one time of day to another. One study has been gathering data systematically and in sufficient quantity to demonstrate the profound effect of timed drug treatment on the experimental animal. The study has been able to relate for the first time in a systematic manner the drug resistance-susceptibility rhythm to a physiologic variable such as that seen in bone marrow and gut epithelium, as well as in a number of other organs, especially as it relates to DNA synthesis in normal and tumor-bearing animals. Such experimental data are a necessary step to the concept of cancer chronochemotherapy in humans. The study plans to identify susceptibility-resistance rhythms to several clinically effective drugs in current regimens. The demonstration that rhythms in DNA synthesis in certain organs and tissues could be synchronized to dosage schedules might ultimately prove to be important as a practical way of synchronizing the same rhythms prior to clinical radiotherapy or chemotherapy.

Toxin Conjugates as Potential Inhibitors:
It has been demonstrated that a mushroom toxin at low concentrations specifically inhibits mammalian RNA polymerase II. A current study proposes to synthesize and characterize the conjugates of macromolecules and to use the conjugates as probes to study molecular mechanisms of antibody production, hormone action and mitogenesis. By studying toxin-peptide hormone conjugates and by the subsequent study of the specific inhibitory potential of these, this research may contribute significantly to a re-evaluation and elucidation of the mechanisms by which peptide hormones interact with specific cells. Conjugates with steroid hormones are also anticipated. Since the steroids are specifically recognized, taken up, and transported to the nucleus of specific receptor cells, it may prove possible to kill specific target cells using steroids as toxin carriers.

Aberrant Gene Expression During Chemotherapy:
An enzyme necessary for metabolism of certain drugs has been detected in T lymphocytes but not in B lymphocytes following blast transformation. This suggested the possibility of using chemical inducers to activate the genes for specific enzymes in tumors prior to chemotherapy, thereby enhancing the therapeutic response. The possibility of preconditioning a tumor so that it is more sensitive to chemotherapy is a novel and important strategy, and one study has provided evidence for a differential change following therapy with a cytidine analog. The results obtained with changes in levels of glucocorticoid receptor are more difficult to interpret, but it is of interest that these

serial changes have been observed during in vivo emergence of tumor resistance to corticosteroids. An in-depth study of the adult and embryonic isozymes in neoplastic cells and of the changes in isozymic profiles in animal tumors following treatment with antimetabolite therapy may lead to preconditioning procedures to alter resistant tumors into a more susceptible form.

Studies on Treatment of Cancer Dissemination and Metastases:
One group of researchers has found that 5-iodo-deoxyuridine possesses significant anti-metastatic activity in mice bearing the Lewis lung (3LL) tumor. The drug is well tolerated and not directly cytotoxic. Treatment with drugs followed by surgery of the clinically detectable primary tumor reduces the number of metastases to 15% of controls. Clear synergism was observed when two immunostimulants having complementary modes of action were combined in the 3LL system. Further studies should provide a greater knowledge of basic biological aspects of the complex problem of cancer dissemination and metastasis and should lead to the characterization of new combined modalities of treatment of metastasis formation for more effective clinical care of patients.

Future Program Emphasis:
A major problem in taking maximum advantage of cell growth characteristics is that currently known techniques are limited in their applicability to an individual patient or to a specific tumor. They cannot provide a quick estimate of the cell kinetic status of tumors under treatment, especially solid tumors. The differences in response between susceptible and resistant tumors emphasize the importance of other factors associated with pharmacokinetics, drug metabolism, distribution, and cell uptake. This area of research will receive greater emphasis since it takes advantage of cell characteristics other than the simple proliferative parameters, and tends to bypass some of the limitations of therapeutic approaches based on existing rationales.

Remarkable advances have been reported recently in our understanding of the biochemical events within the cell at the molecular level during proliferative stages. There is a close relationship between such processes and the rationale for the design and application of chemicals for effective control of cancer. Observations concerning the RNA-dependent DNA polymerase appear to be particularly promising in this respect. Evidence has been obtained that there are RNA sequences in human leukemias and sarcomas which are homologous to the RNA in mouse leukemia virus, and sequences in human mammary tumors which are homologous to mouse mammary tumor virus. It has been postulated that reverse transcriptase is necessary for infection and transformation of cells by RNA tumor viruses. If the polymerase activity is essential for maintenance of the transformed state, there exists a rationale and a basis for selective chemotherapy. Relapse after a remission may be caused by re-infection or re-induction of new normal cells. Hence, compounds which inhibit reverse transcriptase would be of value in therapy. Compounds known to be inhibitory for polymerases, e.g., rifampicins and streptovaricins, are of particular interest. Several other groups of compounds have been identified which appear to have the desired characteristics.

Increased understanding of the role of chemical structure in the inhibition of RNA viral DNA polymerases, viral multiplication, and tumor growth has focused interest on the synthesis of antiviral agents for antitumor testing. One area

of prime synthetic and biochemical interest concerns analogs of daunomycin, adriamycin, tilorone, and distamycin A. The existence of enzymes which catalyze RNA-directed DNA synthesis suggests the possibility that studies with specific inhibitors of such enzymes may ultimately lead to the design of clinically useful anticancer agents.

Another area of prime significance is prevention of cancer cell dissemination; to achieve this goal, it is necessary to determine the relative importance of the various components of the metastatic process. An experimental therapeutic model in mice implanted with Lewis lung carcinoma has been developed which serves as a basis for the selection of drugs effective in retarding or preventing the spread of tumor cells from the primary sites. The concept has been advanced that a form of limited disseminated intravascular coagulopathy may be produced by metastasizing tumor cells in order to facilitate and promote their ability to colonize within other organs. Additional studies will involve various therapeutic attempts to interfere with the process of metastasis formation by combination surgery and/or chemotherapy with or without anticoagulant therapy.

Future program emphasis in the Drug Development Program will relate specifically to development of new modes of treatment of solid tumors. Major limitations to chemotherapy in this group of tumors are (1) permanently resistant variant cells, and (2) resting tumor cell resistance that is not inherent or necessarily permanent. Both types of resistance are most frequently encountered in a relatively large, slow-growing tumor mass. A third limitation to chemotherapy is that tumor cells, because of their anatomic placement, frequently receive less than average drug exposure because of the 'sanctuary' problem; e.g., chemotherapy of intracranial central nervous system tumors depends on the capacity of the drug to cross the blood-brain barrier. Further research must be directed toward an understanding of the mechanisms of resistance and susceptibility, particularly with respect to establishment of laboratory techniques, in vitro or in vivo, for identification of the most effective chemotherapeutic agents for a specific type of resistant tumor.

OTHER ACTIVITIES

During the past year, the Drug Development Program supported an International Conference on the Rational Synthesis of Chemotherapeutic Agents. This conference, held in Milan, brought together scientists from the fields of chemistry, biochemistry, molecular biology, and biology to exchange ideas on prospectives in the field of cancer chemotherapy and to explore new approaches in the area of search for rational synthesis of new anticancer compounds.

This year, the Drug Development Program will also provide support for the 1976 Gordon Research Conference on Cancer. The conference topic will be Basic Science and Chemotherapy. The topics will cover discussions on current research on cellular properties pertaining to action of chemotherapeutic agents, mode of action, design of antineoplastic drugs, and clinical applications of chemotherapy.

The Drug Development Program maintains liaison with all related programs within the Division of Cancer Research Resources and Centers, and with other National Cancer Institute divisions. Activities of the grant-supported program are coordinated with contract-supported collaborative activities of the Drug Research and Development segment of the Division of Cancer Treatment. Special cognizance is maintained of program efforts supported by the two funding mechanisms to assure coordination of total research effort in the Treatment Thrust. Thus, the investigator-initiated, peer-reviewed research segment remains a separate, but contiguous part of the total drug evaluation chemotherapy research at the National Cancer Institute.

A notable feature of drug development is the interplay and blend of empiricism and rational approach based on theoretical science. This applies to the discovery and application of new therapeutic agents. Theoretical science has made many contributions to the rapid and continuing improvement in the way in which drugs can be used more effectively. Considerations such as the basis for selective toxicity, the increasing influence of pharmacokinetics, the explanation of species variations in drug metabolism, structure-activity correlations for drug efficacy and for drug toxicity, and the understanding of cell kinetics in terms of its molecular biology must remain the principal targets of investigation by scientists in cancer research.

RADIATION PROGRAM

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HISTORY AND CONTENT OF PROGRAM

The Radiation Program emerged as an identifiable entity in the National Cancer Institute in the 1960's, and the program has maintained a steady rate of growth. Since 1972, when a second program director was added, the program has been jointly administered by a permanent Program Director for Radiation Biology and Physics and by a rotating Program Director for Radiation Therapy. The latter role is customarily filled by a Radiation Therapist on sabbatical leave from an academic position. The current Program Director is on leave from the University of Louisville.

In FY 1976 the overall program budget is about $29 million, split into three approximately equal portions for traditional research grants (primarily radiation biology), specialized Radiation Research Centers (basic and clinical), and High LET (linear energy transfer) Radiation Research Centers. These latter two areas were selected for emphasis at the beginning of this decade and have been developing well. The High LET Center support doubled each year over the period 1972-1975. In 1976 this rate understandably slowed down but still remains considerable.

RESEARCH ACCOMPLISHMENTS

Clearly the most exciting and potentially the most promising work in radiation therapy research today is in the High LET area. Currently the NCI is supporting, within the United States, four programs aimed at a randomized clinical trial of fast neutrons, one for pions and one for heavy ions.

The four neutron efforts are at: (a) the University of Washington-Seattle (Parker et al., 1975); (b) Texas A&M in conjunction with the M.D. Anderson Hospital (Hussey et al., 1975); (c) the Naval Research Laboratory in conjunction with George Washington University (Rogers, 1975); and (d) the National Accelerator Laboratory outside Chicago. At the first three of these institutions observations have been made on tumor responses and normal tissue tolerance in over 500 patients. Phase I trials on a variety of human cancers will continue concurrent with studies on normal tissue tolerances, optimum doses, and patterns of treatment application. Cooperative clinical trials on head and neck cancer, glioblastoma multiforme, and carcinoma of the uterine cervix are scheduled to commence in FY 1976 and FY 1977.

Preclinical work is underway with pions at Los Alamos Scientific Laboratory in conjunction with the University of New Mexico (Kligerman et al., 1975) and with heavy ions at the Lawrence Berkeley Laboratory of the University of California (Raju et al., 1976). Randomized clinical trials are still a few years away.

Proton beam therapy at the Harvard Cyclotron in conjunction with the Massachusetts General Hospital is currently in the preclinical trial stage (Robertson et al., 1975).

Correlated basic research programs in physics and radiobiology to support the clinically oriented studies are being promoted. Medically dedicated accelerators for placement in research-oriented medical centers are being developed. The clinical assessment of fast neutron therapy is being intensified through the addition of new programs and increased support (where performance justifies) of existing ones.

Clinical trials using proton irradiation, helium ion irradiation, and pi meson irradiation are being mounted. More intensive, long-term commitment of administrative and financial support for the particle therapy research program is being sought.

In radiation research involving conventional photon radiation, interest in the past few years has greatly increased in the combination of hyperthermia and radiation (Harisiadis et al., 1975). In some circles it is being called the "poor man's High LET radiation" since some postulated underlying mechanisms are similar.

Other areas of combined modality research include radiation and surgery, radiation and chemotherapy, and radiation and immunotherapy.

Studies combining surgical irradiation include the use of:

1. Pre-operative, post-operative, or intra-operative (interstitial therapy) irradiation;
2. Surgery or irradiation alone for the primary and irradiation or surgery for lymph node metastases;
3. Less than conventional surgery followed by irradiation for residual subclinical disease;
4. Surgical staging procedures done prior to irradiation.

In Radiation-Chemotherapy studies the use of new drugs and their sequencing with radiation therapy is a subject of intense study. Since metastatic disease is the major cause of death from cancer its control is a great challenge. Combination chemo-radiation therapy offers a possible solution to at least part of this challenge. Control of local regional disease may be enhanced as well. Late effects of these combinations also require elucidation. The use of drugs as radiation sensitizers or potentiators (Kal et al., 1975) is in this same general area of research.

Studies in combined radiation therapy and immunotherapy have been undertaken. Milas et al. (1975 a,b) and Suit et al. (1975) have employed C. parvum and C. granulosum as adjuvants to irradiation of murine fibrosarcomas. Systemic treatment with these organisms greatly augmented the therapeutic effectiveness of local irradiation, in particular when given prior to irradiation. The incidence of pulmonary metastases was less in the animals receiving the adjuvant immunotherapy.

REFERENCES

Harisiadis, L., Hall, E.J., Kraljevic, U. and Borek, C. "Hyperthermia: Biological Studies at the Cellular Level," Radiology 117(2):447-452, Nov. 1975. (CA-12536)

Hersey, D.H., Fletcher, G.H. and Caderao, J.B. "Preliminary Report of the MDAH-TAMVEC Neutron Therapy Pilot Study," Radiation Research, Biomedical, Chemical and Physical Perspectives, p. 1106, Editors O.F. Nygaard, H.I. Adler and W.K. Sinclair, Academic Press, N.Y., 1975. (CA-12542)

Kal, H.B., Hatfield, M. and Hahn, G.M. "Cell Cycle Progression of Murine Sarcoma Cells after X-Irradiation or Heat Shock," Radiology 117:215-217, 1975. (CA-04542)

Kligerman, M.M., Dicello, J.F., Davis, H.T., Thomas, R.A., Sternhagen, C.J., Gomez, L. and Peterson, D.F. "Initial Comparative Response of Experimental Tumors to Peak Pions and X-Rays," Radiology 116(1):181-182, July 1975. (CA-14052)

Milas, L., Withers, H.R. and Hunter, N. "C. granulosum and C. parvum induced Augmentation of the Radiocurability of a Murine Fibrosarcoma," Proc. Am. Assoc. Cancer Res. 16:154, 1975a. (CA-06294)

Milas, L., Withers, H.R. and Hunter, N. "Combination of Local Irradiation with Systemic Application of Anaerobic Corynebacteria in Therapy of a Murine Fibrosarcoma," Cancer Res. 35:1274-1277, 1975b. (CA-06294)

Parker, R.G., Gerdes, A.J. and Berry, H.C. "Clinical Experience of Therapy with Cyclotron Neutrons at the University of Washington," Radiation Research, Biomedical, Chemical and Physical Perspectives, p. 118, Editors O.F. Nygaard, H.I. Adler and W.K. Sinclair, Academic Press, N.Y., 1975. (CA-12441)

Raju, M.R., Blakely, E., Howard, J., Lyman, J.T., Kalofonos, D.P., Martins, B. and Yang, C.H. "Human Cell Survival as a Function of Depth for a High-Energy Neon Ion Beam," Radiation Research 65(1):191-194, Jan. 1976. (CA-15184)

Robertson, J.B., William, J.R., Schmidt, R.A., Little, J.B., Flynn, D.B. and Suit, H.D. "Radiobiological Studies of a High-Energy Modulated Proton Beam Utilizing Cultured Mammalian Cells," Cancer 35(6):1664-1667, June 1975. (CA-13311)

Rogers, C. "The Middle Atlantic Neutron Therapy Trial," Radiation Research, Biomedical, Chemical and Physical Perspectives, p. 1092, Editors O.F. Nygaard, H.I. Adler and W.K. Sinclair, Academic Press, N.Y., 1975. (CA-17465)

Suit, H., Sedlacek, R., Wagner, M. and Orsi, L. "C. parvum and Local Irradiation as a Treatment of a C_3H Fibrosarcoma," Proc. Am. Assoc. Cancer Res. 16:196, 1975. (CA-13311)

PROJECTIONS

In the traditional research grant portion of the Radiation Program, a moderate rate of growth is anticipated, comparable to that of the past six years. This projection would have to be markedly revised upward should the U.S. Energy Research and Development Agency (formerly, Atomic Energy Commission) heavily cut back on the excellent radiation biology and physics work it has supported for many years.

Moderate growth is also anticipated in the Radiation Research Center portion of the program, resulting in one or two additional centers (basic or clinical per year. The size of the field is such that this projection can be made wit considerable confidence.

In the High LET Radiation Center portion of the program, it is quite difficul to make such a confident prediction. This uncertain state of affairs results from the great promise of research in this area and the great cost of such research. Present estimates project a cost of approximately $250 million for High LET radiation over the next ten years. This sum would allow development of optimized equipment for randomized clinical trials of neutrons, protons, pions, and heavy ions and for execution of such trials after selection of abc four centers for each of these different particles (except neutrons, which ha already entered into clinical trials at five centers funded by NCI). The jus tification of such large expenditures rests on the expected potential of such radiations to save upwards of 60,000 lives yearly.

OTHER ACTIVITIES

In addition to funding research and development efforts, the Radiation Progra engages in various other activities aimed at promoting and stimulating radia- tion research.

In April 1975, an International Symposium on Cancer Therapy by Hyperthermia a Radiation was held in Washington, D.C. in conjunction with the American Colle of Radiology (ACR) and the University of Maryland School of Medicine. More than 100 people attended and, as a direct result, there has been a very noti able increase in the number and quality of applications to the NCI in this ar This has been our most successful effort in stimulating research in an area which clearly was in need of such stimulation.

In October 1975, a meeting was held in Key Biscayne, Florida, on Recent Prog in High LET Radiation Therapy. This meeting was jointly sponsored with the and was by invitation only. It served as a prelude to the International Con ence on Particles and Radiation Therapy to be held in Berkeley, California, September 1976. This latter meeting is open to the general public; 300-400 attendees are expected.

The Radiation Program has available to it an unofficial advisory group (the Committee on Radiation Oncology Studies) comprised of eminent radiation biol gists, physicists, and therapists. This group, which receives grant support, initiates projects it considers important to the Radiation Program and responds

to specific requests for assistance. The major effort of the CROS in FY 1976 has been formulation of what is called the Research Plan; this is a review of the state of radiation research, which contains recommendations for direction of future progress. This document, which will be updated periodically, will prove invaluable in the years to come as guidance for the Radiation Program as well as for potential grantees.

CANCER CENTERS BRANCH

William L. Roberson, M.D., Chief

HISTORY AND PROGRAM ACCOMPLISHMENTS

In response to the need for new knowledge through scientific research and rapid translation of the findings into coordinated care for cancer patients, the National Cancer Institute (NCI) activated in 1961 a Cancer Research Centers Program. This program was designed to provide grants for the support and development of cancer complexes which could engage in clinical and basic research, discover ways to improve the diagnosis and treatment of cancer patients, train an effective cancer cadre for the future, and hasten the upgrading of cancer care in surrounding areas. Cancer centers have increased significantly in number during the present decade and diversified in function according to needs and the special facilities and personnel available in differing areas of the country. In recent years, there has been increasing emphasis on centers of broad scope which could provide a comprehensive, multidisciplinary attack upon cancer problems. The National Cancer Act of 1971 and the National Cancer Act Amendments of 1974 gave further stimulus to this trend by providing for the establishment of additional cancer centers. Because they serve as foci for a variety of high priority thrusts in the attack upon cancer, these centers have assumed an increasingly important and visible role in the intensified strategy of the National Cancer Program.

In April 1973, the Special Programs Branch, which had administered cancer center activities, was abolished and the Cancer Centers Branch established. During the present fiscal year, 1976, there have been additional organizational changes. The Cancer Centers Branch is currently one of the three branches of the Centers and Treatment Program and its function is to develop, direct and administer a program of exploratory grants and core support grants for comprehensive and specialized cancer centers; evaluate the effectiveness of the Cancer Centers Program; identify as components of center programs the cancer-related basic and clinical projects in the Biological Research Program and the Diagnosis and Treatment Branch; coordinate branch activities with related activities in other Divisions of the Institute; review and present grant applications and provide special reports as required to the National Cancer Advisory Board; and maintain liaison with appropriate Federal and non-Federal organizations, institutions, and scientists.

Guidelines for Cancer Centers were developed during 1972 with advice from the National Cancer Advisory Board and the President's Cancer Panel. These guidelines, published in 1973, outline the procedures to be followed in the establishment of Cancer Centers and describe the characteristics of Comprehensive and Specialized Centers. The guidelines were revised this fiscal year (1976) to include an emphasis on the Cancer Center Support (Core) Grant. The purpose of a Core grant is to provide a mechanism to support those elements of a cancer center that are required for the planning, development evaluation, administration, and maintenance of an active and unified cancer center in order to consolidate and focus cancer-related activities in a single administrative and programmatic structure. Through this mechanism, support may be provided which

can contribute to the stability of the center, to administrative and programmatic control of center activities, and to fiscal accountability and responsibility.

The Core grant provides support for professional staff, centralized services and resources, and shared equipment and developmental projects. Day-to-day research and component cancer center functions are funded through other funding mechanisms; e.g., research project grants, education grants, and contracts, as well as non-NIH sources of support.

Proposals for Core grants undergo competitive scientific and technical peer review. An important criterion is a record of cancer activities of high quality and an administrative organization that will promote and conduct a concentrated attack on the cancer problem.

In FY 1976, 42 Core grants were awarded, for approximately $36.1 million. Included are five new grants; six renewal grants; ten supplemental grants; and 31 continuation grants. The total amount of Core support requested for competing grants in FY 1976 totaled $26 million, and $13.2 million was recommended by the NCAB. Because of the very heavy workload it was necessary for Center Branch staff to assist in the review process, including organizing site visits, serving as executive secretary, and preparing summary statements.

Exploratory Grants, sometimes referred to as planning grants, have been utilized by the NCI since FY 1966. The majority of these applications have been stimulated by NCI staff, especially in the early years. They are peer reviewed by a Special Review Committee and a site visit is almost always made as a part of the review process. Exploratory Grants include support for planning new cancer centers, expansions, and modification or strengthening of existing centers. Grant funds are not limited to cancer center activities but may be used for the planning of a number of cancer programs, facilities, or resources. This has been especially true in the field of radiation oncology. Feasibility studies are included in the concept of planning but may not be used to provide preliminary research data for research grant applications. Exploratory Grants allow an institution to do a careful analysis of the various alternatives available to develop a cancer program consistent with local needs and organizational structures as well as to contribute to the goals of the National Cancer Program. In FY 1976, the Centers Program awarded 34 Exploratory Grants at approximately $2.7 million in total costs. These figures include continuation, new, renewal, and supplemental grants. Exploratory Grant applications requesting $4.3 million were reviewed, resulting in 26 applications being recommended for approval at $2.9 million in total costs.

PROJECTIONS

Cancer Centers which have been receiving support through one or more large Center Grants ("Umbrella") have been requested to separate their programs into Core plus an appropriate number of clinical and/or basic program projects and regular research projects. This will allow for a more thorough review of all components and better fiscal accountability. The conversion process should be completed by the end of the 1976 calendar year. The administration of clinical

program project research grants is now the responsibility of the Diagnosis and Treatment Branch. Basic science program project research grants are administered by the appropriate scientists in the Biological Research Program. As a result, relatively little actual research will be funded from the Core Grants. That research which is supported will be limited to developmental projects.

Developmental or "seed" funds will provide the center flexibility in developing new programs or strengthening existing programs to correct areas of weakness within the center. Requests for funds in this area should be documented in the grant application. These funds may be used for the inauguration of new projects by providing salary support for recruiting promising investigators and other professional personnel, together with the appropriate personnel, equipment, supplies, or hospital costs needed to begin their work. Such new projects must be described in detail. Investigators receiving support in this manner are required to seek support for continuation of these developmental projects through the usual competitive funding mechanisms. Core Grant funds may not be used for more than three years for any one developmental project.

OTHER ACTIVITIES

One of the characteristics of a Comprehensive Cancer Center as determined by the National Cancer Advisory Board is the maintenance of a statistical base for evaluation of the results of its program activities. NCI staff is continuing to work with the Centers in this regard. The first meeting of Epidemiology-Statistical (Epi-Stat) Directors was held in October 1975, in Philadelphia. The meeting was funded by an NCI conference grant and was attended by representatives from the 17 comprehensive centers. In addition to the Epi-Stat Directors, there were in attendance a number of statisticians, epidemiologists, and computer specialists - all involved in the development of the data needs of the centers. The conference was successful to the extent that the attendees elected to organize and probably will meet annually if funds are available. Also, the following committees were formed: Evaluation Methodology, Data Base Needs and Definitions, Quality of Life Measurement, Collaborative and Etiologic Studies, and Epidemiological, Statistical, and Computer Training. The Committees intend to exchange information and ideas and have final reports available in October 1976.

A major activity of the Epi-Stat Directors during the past year has been the development of the necessary means to supply the data designed to meet the needs of the International Cancer Research Data Bank (ICRDB) Program as well as the minimal needs of the centers themselves. The data reporting system that is developed will be designed to be compatible with the NCI Surveillance, Epidemiology and End Results (SEER) System.

CANCER RESEARCH FACILITIES CONSTRUCTION PROGRAM

Donald G. Fox, Ph.D., Chief

HISTORY AND CONTENT OF PROGRAM

The Cancer Research Facilities Construction Program was initiated in late September 1971 with a public announcement of the availability of limited grant funds to create additional cancer research facilities. The essence of this announcement is set forth in the following paragraph:

> In accordance with the President's call for an expanded, intensified, and coordinated cancer research program, and under authority provided by the Congress in the fiscal year 1972 appropriation act, the National Cancer Institute is initiating a program of grant-supported construction of cancer research facilities. The intent of this program is to create new physical resources for cancer research through Federal participation in the cost of new construction and renovation. Support may be provided for the construction of facilities such as basic research laboratories; clinical research facilities; animal facilities; and basic associated core, administrative, laboratory and service space. In all instances, the facilities proposed must be intended for expansion of cancer research for at least 20 years. A purpose of this program, in addition to strengthening research capabilities at existing cancer centers, is to develop new strong multidisciplinary cancer efforts in regions of the country where they do not exist. Thus, in the consideration of applications some attention will be given to geographical distribution as well as to the relation of proposed construction to existing centers of excellent cancer research.

Justification of construction funds has been, and will continue to be, in terms of new cancer research programs and safe facilities for accomplishing biohazardous cancer research. The primary review and evaluation of applications has been essentially scientific, considering such matters as scientific merit of the proposed program(s), the technical competence of the applicant institution staff, the intellectual environment of the applicant institution, and its scientific, fiscal, and administrative capabilities. In addition, other criteria have been considered, such as the geographical location of the applicant institution, the applicant institution performing an essential role in the National Cancer Program, the promptness with which construction can be started, space requested commensurate with projected program scope, essential minimal facilities for those institutions with an undeveloped cancer research program, net space utilization of 60 percent or more, reasonable cost per gross square foot, acceptable design criteria, etc.

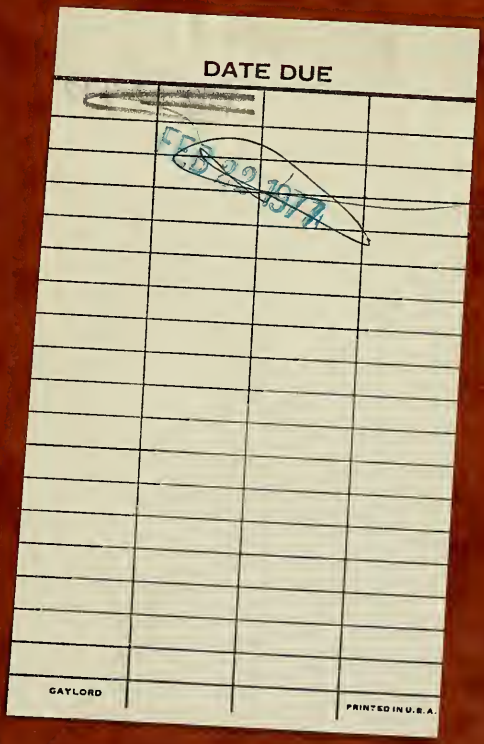
During the first three quarters of FY 1976, 17 construction grant applications were approved by the National Cancer Advisory Board in November and March. Seven of these projects were also recommended for funding at the November

meeting: Massachusetts Institute of Technology, State University of New York (Stonybrook), Memorial Hospital, Sloan-Kettering Institute, University of Hawaii, Rockefeller University, and Harvard University. In addition, a supplemental funding request from the Sidney Farber Cancer Center was approved for payment. Five projects were recommended for funding at the March meeting: University of Michigan, University of Wisconsin (2), Northwestern University, and the Fred Hutchinson Cancer Research Center. In addition, supplemental funding requests from the Albert Einstein College of Medicine and The Johns Hopkins University were approved.

By the end of this year, $158,686,000 will have been awarded under the Cancer Construction Grant Program since its beginning in FY 1972, $20 million during FY 1976.

RECENT ACCOMPLISHMENTS

1. Issuance of Construction Regulations in the Federal Register. The issuance of our regulations by this formal procedure required the review and approval of all Federal Agencies and of interested non-Federal groups.
2. Publication of a technical handbook to describe the detailed architectural and engineering requirements of cancer research facilities. The handbook draws together all of the scattered requirements including new requirements in biohazard containment, energy conservation, and earthquake resistance. The handbook serves as NCI's major input to the Grantee's design team.
3. Of the 67 construction grants awarded, 32 projects are under construction and ten others have been completed.



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